DEVELOPMENT AND STANDARDISATION OF POLY HERBAL GEL AND CLINICAL EVALUATION OF ITS HAIR GROWTH STIMULATION

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DEDICATED TO MY BELOVED PARENTS

"GRATITUDE IS THE ATTITUDE OF NOBLE BEING"

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Introduction

1. INTRODUCTION

India has a good climatic condition and provide more natural source, this promise to cure and prevent almost most of disease .Being in a stressful life style, most of the people experiences hair loss and they feel themselves less comfortable among others, As a way of finding a cure to it, they started using many synthetic lotions and many other preparations including hair transplantation technique. As the peak of their curiosity, they pick the most harm full treatment, it gives the person not only hair him/her needs, but also gifts him/her with may health problem and most shocking turns to be lethal. This work under the topic of "**Hair growth; Herbs prove promising in hair growth**.

Now a day the diffuse hair loss is a common problem among the people between the of age 18 to 50 due to various health disorder, synthetic drug side effect, stress, malnutrition. The herb promise to cure the problem but single plant some time inadequate to produce quick benefit that Way to produce adequate effect by combining the plant by advance developed technology to provide more efficient effect.

Herbal medicine is defined as the branch of science in which plant based formulations are used to alleviate the diseases. It is also known as botanical medicine or phytomedicine. In the early twentieth century herbal medicine was prime healthcare system as antibiotics or analgesics were not available. With increasing use of allopathic system of medicine, herbal medicine gradually lost its popularity among the people and the success of allopathic system was based on the fast therapeutic actions of synthetic drugs.

1.1. HAIR LOSS

Hair loss is known that diseases due to the various causes like the Nutritional deficiency, Hormonal imbalance, autoimmune disorder, Stress and Chemotherapy.

1.1.1. Nutritional Deficiency[1]

- **Protein deficiency** like the atrophy of hair follicles in protein-energy malnutrition diseases such as Marasmus and Kwashiorkar, Both diseases cause fine or brittle hair and alopecia instigated by telogen effluvium.
- **Zinc deficiency** may result in dry and brittle hair and diffuse or patchy alopecia that worsens over time.

- **Selenium deficiency** may causes alopecia and a hypo-pigmentation of the hair and skin.
- **Vitamin A deficiency** may cause excessive keratinization of the hair follicles, presenting keratin plugs.
- A lack of **biotin** is a severe, even lethal deficiency in foetuses, but in those infants who do survive, biotin deficiency's clinical signs include universal alopecia.
- The most common diet deficiency, **iron deficiency**, may also result in alopecia.

1.1.2. Hormone imbalance

- **Androgenetic alopecia** [1,2,3]

In consequence of circulating testosterone being converted to Dihydrotestosterone (DHT) by the enzyme 5α-reductase. Although DHT may support beard, chest, and axillary hair growth, it has a very different effect when it binds to the androgen receptor on certain hair follicles of the scalp. The binding shortens the growth phase of the hair follicles and lengthens the telogen phase, resulting in finer, lighter, and shorter hairs over time. Androgenetic alopecia is more common among men and follows a trademark pattern of balding beginning with a receding bitemporal hairline and then a loss of hair at the crown of the head.

1.1.3. Autoimmune Disorder [4,5,6,7]

- Alopecia areata is another relatively common hair disorder occurring in healthy men and women.
- Alopecia areata can cause hair loss ranging from small, patchy areas of baldness on the scalp to a complete loss of hair on the entire body. A lymphocytic infiltrate around hair follicles; the infiltrate consists mostly of helper-T cells.
- Activated CD4 and CD8 lymphocytes are likely key players in the pathogenesis of alopecia areata.

- Alopecia areata patients may experience full hair re-growth during their lifetime, but many lose hair permanently.

1.1.4. Psychosocial effects of hair loss [1]

Although hair loss is often a clinical manifestation of a serious internal stress or illness, hair loss itself is sometimes the cause of the distress.

1.1.5. Drug induce hair loss [8]

Drug can induce hair loss, but initially it is difficult to identify. The noxious substance may affect the growth phase of the hair growth cycle. There are two mechanisms of drug-induced alopecia. They are as follows,

a) Direct effects

Include anagen growth interruption, precipitation of catagen, and disturbed keratinization, resulting in hair shaft damage.

b) Indirect effects.

Indirect effects include causing a systemic disease (hypothyroidism or zinc deficiency) or a severe skin disease (lichenoid eruption or toxic epidermal necrolysis) of which alopecia is a feature.

Scalp follicles are in differing phases of the hair cycle and are randomly scattered over the scalp. Almost 90% of scalp follicles are in anagen, 10% in telogen, and 1% in catagen. Follicles are susceptible to noxious agents, usually when they are actively growing. During the anagen phase, the mitotic activity of the hair matrix is so high that it can be compared with the most actively kinetic tissues of the body, namely bone marrow and mucous membranes. For this reason anagen hair matrix is highly susceptible to noxious events.

- **Anticancer drug induced hair loss**

Adjuvant and metastatic Chemotherapy regimens often cause hair loss as a side effect. In studies conducted on breast cancer patients undergoing Chemotherapy, hair loss was considered traumatizing and distressing by patients, and one study found women thought the hair loss was more difficult than losing a breast. Hair loss as a result of chemotherapy is seen as a loss of privacy because it revealed to others that the patient is being treated by chemotherapy. Patients attributed an altered sense of self and a

1.2. STRUCTURE OF HAIR [9,10]

Hair follicles have a cyclic activity characterized by alter Nance of periods of hair shaft production and of resting; The hair cycle consists of different stages.(Fig. 1.1)

1.2.1. The hair follicle

The hair follicle (Fig. 1.3) is divided in two portions:

- The upper follicle, permanent
- The lower follicle, dynamic.

The hair bulb, which is the deep bulbous portion of the hair follicle that surrounded the dermal papilla, contains the hair matrix which produces the hair shaft and its sheets (Fig.1.5).

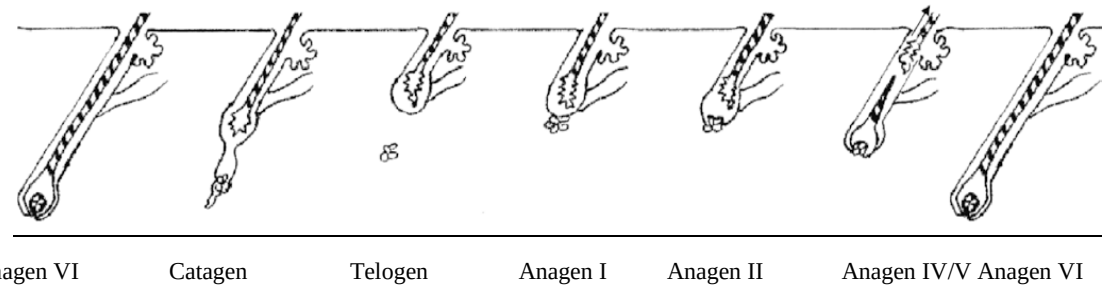


Fig.1.1 Stages of hair growth cycle

1.2.2. Hair Growth Cycle [11]

The hair growth cycle involve three stages.(Fig. 1.2)

- **Anagen** -hair shaft production which lasts 2–6 years

During the growth phase of the hair cycle, the hair shaft is created from rapidly proliferating matrix cells.

The hair follicle actively produces the hair shaft. This phase is the longest of the hair cycle and its duration determines the length of the hair shaft. Due to the high mitotic rate of follicular matrix cells, the anagen phase is very sensitive to noxious insults.

- **Catagen**-transitional phase which lasts 2–3 weeks

It is characterized by apoptosis of the hair matrix cells and involution of the lower part of the follicle. During catagen, the hair bulb migrates from the

hypodermis to the mid dermis.

- **Telogen**- Resting phase lasts about 3 months

During this phase hair shaft production is absent and the hair bulb is completely keratinized. Telogen phase, in the scalp follicles, lasts about 3 months but it is considerably longer in other body regions such as the lower limbs. The hair shaft remains anchored to the follicle during telogen. Hair shedding (teloptosis/exogen) usually occurs when the follicle re-enters a new anagen phase. In some follicles teloptosis (Fig.1.4) occurs before the end of telogen and the follicle remains empty for a certain period (kenogen). This may be due to a premature teloptosis or prolonged telogen duration.

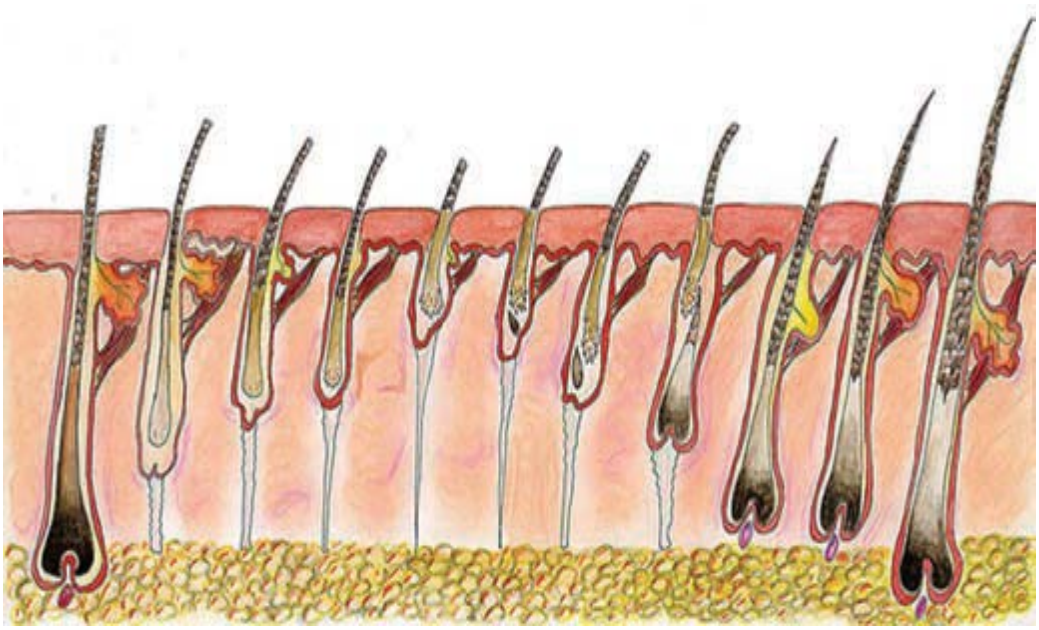


Fig. 1.2 Hair Growth Cycle

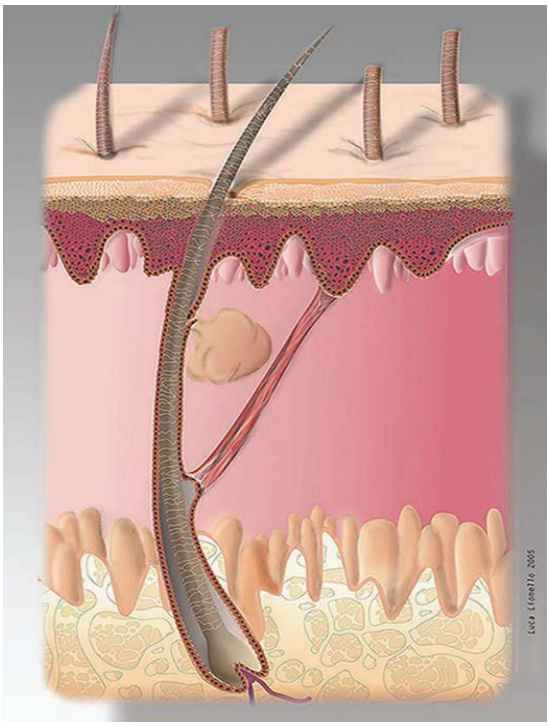


Fig. 1.3 Hair follicle.

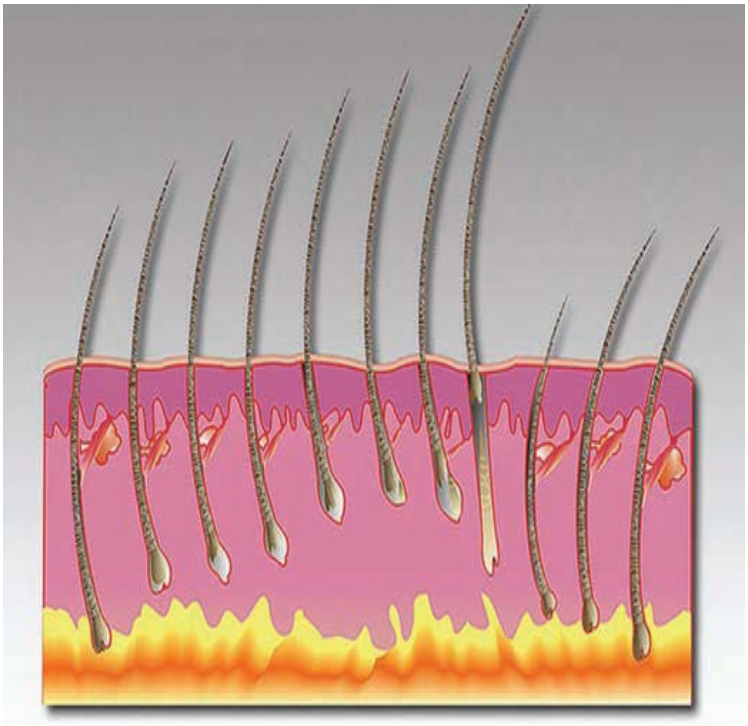


Fig. 1.4 Teloptosis

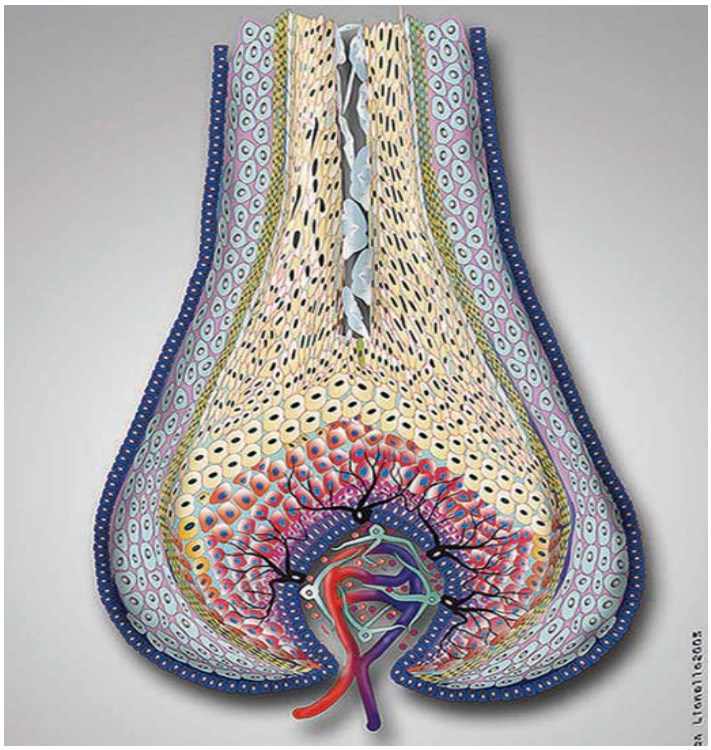


Fig. 1.5 Hair matrix

1.3 HERBAL FORMULATION

Ayurveda is one of the traditional medicinal systems of Indian. The philosophy behind Ayurveda is preventing unnecessary suffering and living a long healthy life. Ayurveda involves the use of natural elements to eliminate the root cause of the disease by restoring balance, at the same time create a healthy life-style to prevent the recurrence of imbalance. Herbal medicines have existed world-wide with long recorded history and they were used in ancient Chinese, Greek, Egyptian and Indian medicine for various therapies purposes. World Health Organization estimated that 80% of the word's inhabitants still rely mainly on traditional medicines for their health care. The subcontinent of India is well-known to be one of the major biodiversity centers with about 45,000 plant species. In India, about 15,000 medicinal plants have been recorded, in which the communities used 7,000-7,500 plants for curing different diseases. In Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. The Ayurvedic literature Sarangdhar Samhita' highlighted the concept of polyherbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. This review mainly focuses on important of the polyherbalism and its clinical significance.

The formulation in *Ayurveda* is based on two principles

a. Use as a single drug and
b. use of more than one drugs,

The latter one is known as Poly Herbal Formulation (PHF). This key traditional therapeutic herbal strategy benefits from the combining of several medicinal herbs to achieve extra therapeutic effectiveness, usually known as polypharmacy or polyherbalism.

Historically, the *Ayurvedic* literature "*Sarangdhar Samhita*" dated centuries ago in 1300 A. D. has highlighted the concept of polyherbalism in this ancient medicinal system. In the traditional system of Indian medicine, plant formulations and combined extracts of plants are chosen rather than individual ones. It is known that *Ayurvedic* herbals are prepared in a number of dosage forms, in which mostly all of them are Poly herbal formulation.[12]

The active phytochemical constituents of individual plants have been well established, they usually present in minute amount and always, they are insufficient to achieve the desirable therapeutic effects. For this, scientific studies have revealed that these plants of varying potency when combined may theoretically produce a greater result, as compared to individual use of the plant and also the sum of their individual effect.[8]

1.4 Herbal Remedies for hair loss

Medicine from the nature is more beneficial than synthetic, Long time use of synthetic agent inversely affect the physiological action leading to increase the adverse effect, that point to view diminish the noxious effect of the substance replacing by alternative system of medicine.

Herbs are promising to treat almost all disease in universe, hair loss more prone to age between 18 to 60.Some plants available for the treatment of hair loss

Table 1.1 List of Plants For treatment of Hair loss

S.no	Biological Name	Family	Part used
2.	*Aloe vera*	Liliaceae	Juice
3.	*Abrus precatorius*	Fabaceae	Seeds
4.	*Allium tubarosam*	Amaryllidaceae	Seeds
5.	*Bacoba monnieri*	Plantaginaceae	Leaves
6.	*Eugenia jambolana*	Myrtaceae	Seeds and fruits pulp
7.	*Eclipta alba*	Asteraceae	Whole plant
8.	*Hybiscus rosa sinencis*	Malvaceae	Leaves
9.	*Legenaria ceraria*	Cucurbitaceae	Leaves
10.	*Phyllanthus emblica*	Phyllanthaceae	Fruits
11.	*Phyllanthus niruri*	Phyllanthaceae	Whole plant
12.	*Trichosanthes cucumerina*	Cucurbitaceae	Leaves
13.	*Trichonella foenugreacum*	Fabaceae	Seeds
14.	*Tridax procumbens*	Asteraceae	Leaves

The poly herbs available in the form of various formulations are hair oil, hair gel, hair cream.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The selection of plant for formulation based on the ability to promote the hair growth and restoring properties many plants with folklore claim for hair growth. Among those plant *Lagenaria siceraria*, *Trichosanthes cucumerina, Tridax procumbens*, has an ethno medical claim in treatment of hair loss. Hence the literature review of the plant was done to find out the nature of scientific evaluations carried out on those plants.

Petchi RR *et al*. (2013) Anti]diabetic and antihyperlipidemic effects [13]

The ethanolic extract of whole plant of *Tridax procumbens* showed marked anti diabetic effect in streptozotocin and nicotinamide induced diabetic male wister rat and also inhibit the streptozotocin induced weight loss and significantly alter the lipid level.

Jachak SM *et al*. (2011) Anti-inflammatory, cyclooxygenase inhibitory and antioxidant activities [14]

The various solvent extract of *Tridax procumbens* arial part showed significant inhibition of rat paw edema at a medium dose of 200mg /kg. The different solvent methanol, ethanol and ethylacetate were used for extraction of the active compound. Among the different extract ethyl acetate was the most active.

Al Mamun MA Hosen MJ *et al*. (2015)*Tridax procumbens* flavonoids promote osteoblast differentiation and bone formation.[15]

The *Tridax procumbens* flavonoid tested on primary mouse calvarial osteoblasts. flavonoids from *Tridax procumbens* showed upregulation of bone hormones mainly osteocalcin. Osteocalcin play a major role in body's metabolic regulation and bone building. Mainly suggested to the patient with bone loss associated disease such as osteoporosis.

Saxena M *et al*. (2013) in-vitro antioxidant activity isolated bioactive compounds from Tridax procumbens Linn.[16]

The causes of many disease mainly cancer induced by free radicals, daily need to remove the free radicals from body by anti-oxidant. The present review shows a phyto-constituent of *Tridax procumbens* possess significant anti-oxidant properties. The methanolic extract of plant further purified isolated by n-butanol soluble part and ethyl acetate soluble p

art were quantified the total phenolic and total flavonoids. The isolated different fraction were employed for *in vitro* anti-oxidant effect by 1,1 Diphenyl,2-Picryl Hydrazyl (DPPH) assay. The n butanol soluble part and ethyl acetate soluble part possess a significant anti DPPH activity while comparable with the Standard ascorbic acid.

Duangmano S *et al*.(2012) Cucurbitacin B inhibits human breast cancer[17]

The growth inhibitory effect of cucurbitacin B on breast cancer cells was assessed by MTT assay. The effects of cucurbitacin B on microtubules morphological structure and tubulin polymerization were analyzed using immunofluorescence technique and tubulin polymerization assay kit, respectively. Proteomic analysis was used to identify the target-specific proteins that involved in cucurbitacin B treatment. Some of the differentially expressed genes and protein products were validated by real-time RT-PCR and western blot analysis. Cell cycle distributions and apoptosis were investigated using flow cytometry Cucurbitacin B exhibited strong anti-proliferative effects against breast cancer cells in a dose-dependent manner

Sathesh Kumar S, *et al*. (2009) Hepatoprotective effect of *Trichosanthes cucumerina* [18]

The methanolic extract of whole plant of *Trichosanthes cucumerina* was evaluated for the hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. Various biochemical parameters like alanine amino transferase, aspartate amino transferase, alkaline phosphatase, total bilirubin, total protein and albumin levels were estimated in serum as well as the glutathione and malondialdehyde levels in the liver were determined. Histopathological changes in the liver of different groups were also studied.

Shah SL *et al*. (2012) Cardioprotective Activity of Methanol Extract of fruit of *Trichosanthes cucumerina* [19]

Cardiac toxicity by doxorubicin was manifested as body weight loss, elevated serum Lactate dehydrogenase (LDH) and serum creatine kinase MB (*CK*-MB), increased ST, QT and QRS complex, reduced blood pressure, and left ventricular function. The methanol extract of T. cucumerina significantly decreased LDH and CK-MB, reduced ST, QT interval and QRS complex, increased heart rate, restored blood pressure, and left ventricular function. Doxorubicin caused liver and kidney necrosis, cellular infiltration, and vascular changes that indicated injury. T. cucumerina (1000 mg/kg) reduced the severity of doxorubicin-induced cardiac damage especially in heart. It is concluded that doxorubicin-induced cardiotoxicity is reduced by pretreatment with methanol extract of fruit of T. cucumerina

Arawwawala LD *et al*. (2010) Gastroprotective activity of *Trichosanthes cucumerina*.[20]

The hot water extract (HWE) of *Trichosanthes cucumerina* possesses significant dose dependent gastro-protective effects in the alcohol model in terms of the length and number of gastric lesions mediated by alcohol, with a maximum effect at 750 mg/kg . The same dose also mediated a significant gastro-protective activity in the indomethacine model. In both models, the

protective effect demonstrated by the hot water extract was comparable with that produced by cimetidine. *Trichosanthes cucumerina* exerts a significant protection against ethanol or indomethacin induced gastric damage. Increase the protective mucus layer, decreasing the acidity of the gastric juice and antihistamine activity.

Thube Smita *et al*. (2009)In-vitro Anthelmintic Activity of Seed Extract of *Lagenaria siceraria*[21]

Preliminary phytochemical screening of the crude extracts revealed the presence of sterols, proteins, carbohydrates, glycosides and saponins. The different extracts exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration. The methanolic extract of L.siceraria caused paralysis of 9 min and time of death of 17 min while benzene extract revealed paralysis of 12 min and death of 22 min against the earthworm *Pheritima posthuma*. The reference drug Piperazine citrate showed the same at 20 and 45 minutes respectively. The traditional use of the seeds of *L.siceraria* as anthelmintic has been confirmed as the different extracts showed good anthelmintic activity in the study.

Vishal R. Mali *et al*. (2010) Cardioprotective effect of *Lagenaria siceraria* (LS) fruit powder in isoprenalin-induced cardiotoxicity in rats[22]

It is concluded that long period (51 days) administration of *Lagenaria sicereria* fruit powder reduced isoprenalin-induced tachycardia and prevented decrease in systolic BP. The cardiotoxic effect of isoprenalin was less in LS pretreated animals. The antioxidant and anti-inflammatory activity of LS appears to contribute in the cardioprotective effect of LS in isoprenalin-induced cardiotoxicity

Prerona Saha *et al*. (2010) Antihyperglycemic activity of *lagenaria siceraria* [23]

The aerial parts of *L. siceraria* methanol extract can be considered as a potent source of antidiabetic agents, which may be attributed to the flavonoid and polyphenolic content of the extract. Additionally, it improves lipid metabolism and represents a protective mechanism against the development of atherosclerosis, and prevents diabetic complications from lipid peroxidation by improving the antioxidant status in experimental diabetic rats.

Schlumbaum *et al*. (2012) A short history of *Lagenaria siceraria* (bottle gourd) in the Roman provinces[24]

Lagenaria siceraria (Molina) is pantropic and displays large variation in fruit and seed shape. Two subspecies are currently recognized, (i) The African *L. siceraria* ssp. *siceraria* and (ii) The Asian *L. siceraria* ssp. *asiatica*. The Asian type of bottle gourd belongs to the earliest domesticated plants in the Americas. In Europe, bottle gourd only appears with some frequency from the Roman period onwards. The paper is the study of ancient DNA (aDNA) and seed morphology of one almost complete bottle gourd fruit from the Roman site of Oedenburg/Biesheim–Kunheim, France (1st century A.D.), and from individual seed finds from the Roman *vicus* of Petinesca–Vorderberg, Switzerland (3rd century A.D.), both recovered from waterlogged layers. Width and length measurements of seeds show large variation.

Kirana H, *et al*. (2008) *Trichosanthes cucumerina* Linn. improves glucose tolerance and tissue glycogen in non-insulin dependent diabetes mellitus[25]

Non-Insulin Dependent Diabetes Mellitus (NIDDM) was induced by administering streptozotocin (90 mg/kg, i.p.) in neonatal rat model. NIDDM animals were treated with aqueous extract of *Trichosanthes cucumerina* (100 mg/kg/day) orally for six weeks. Parameters such as fasting blood glucose, Oral Glucose Tolerance Test (OGTT) and tissue glycogen content were evaluated. Aqueous extract of *Trichosanthes cucumerina* significantly ($P<0.01$) decreased the elevated blood glucose of NIDDM induced rats.

Liyanage *et al*.(2016) Comparative Analysis of Nutritional and Bioactive Properties of Aerial Parts of Snake Gourd (*Trichosanthes cucumerina Linn.*)[26]

The present investigation was carried out to determine the nutritional and functional properties of *T. cucumerina*. Water extracts of freeze dried flowers, fruits, and leaves of T.

cucumerina were evaluated for their total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, α-amylase inhibitory activity, and fiber and mineral contents. Antioxidant activity, TPC, and TFC were significantly higher in leaves than in flowers and fruits**.**

Anbarasu K *et al*. (2011) Antipyretic, anti-inflammatory and analgesic properties of nilavembu kudineer choornam: a classical preparation used in the treatment of chikungunya fever [27]

Ethanolic extract of nilavembu kudineer choornam in inflammation, pain and fever were carried out in animal models to support its actions. Acute toxicity study of ethanolic extract of nilavembu kudineer choornam was performed in mice to fix the effective dose. The antipyretic, anti-inflammatory and analgesic activity Ethanolic extract of nilavembu kudineer choornam was evaluated in brewer's yeast induced pyrexia in rats, carrageenan-induced inflammation in rats and acetic-acid induced writhing in mice model. The present findings revealed that Ethanolic extract of nilavembu kudineer choornam possesses antipyretic, anti-inflammatory and analgesic activity which supports nilavembu kudineer choornam efficacy in chikungunya fever.

Kenoth *et al*. (2011) Steady-state and time-resolved fluorescence studies on *Trichosanthes cucumerina* seed lectin[28]

Steady-state and time-resolved fluorescence spectroscopic studies have been carried out on *Trichosanthes cucumerina* seed lectin. The fluorescence emission maximum of *Trichosanthes cucumerina* seed lectin in the native state as well as in the presence of 0.1 M lactose is centered around 331 nm, which shifts to 347 nm upon denaturation with 8 M urea, indicating that all the tryptophan residues of this protein in the native state are in a predominantly hydrophobic environment.

RATIONALE FOR SELECTION

3. RATIONALE FOR SELECTION

Herbs combine to gather one formulation it gives better curative and therapeutic effect compare when being as a single drug.

Plants not directly used as medicinal purpose, when it processed and formulated as any one of the suitable formulation gives better therapeutic effect by means of dried powder form or extract from the plant with the advance technique.

Gel formulation is a one of the topical formulation and it gives better absorption on the skin and less adverse effect comparable other formulation. When the plant formulated as gel it gives better absorption through skin and gives maximum therapeutic.

Lagenaria siceraria is a small shrub under cucurbitaceae family. It is grow over the tropical and subtropical area and easily available. *Lagenaria siceraria* has various ethno botanical applications and medicinal claims. It fruits have been use vegetables and the ethanomedicinal uses of leaves shows hair growth activity

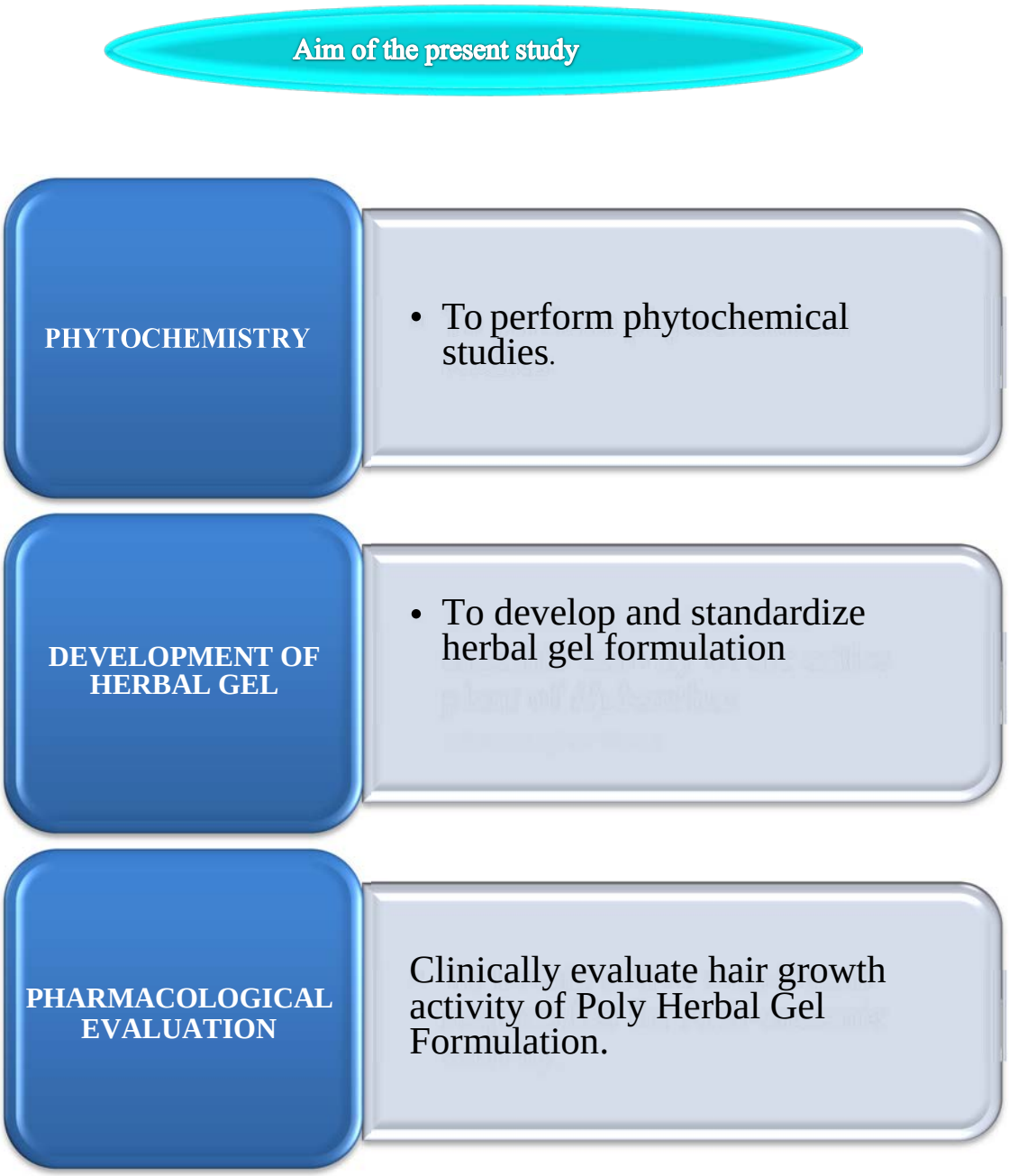
Trichosanthis cucumarina , is a small shrub under cucurbitaceae family. It is grow over the tropical and subtropical area and easily available. Its ethanomedicinal uses shows action on hair growth.

Tridax procumbens is a small weeds procumbent herb, Asteraceae family and easily available through the tropical and subtropical areas. Its leaves ethanomedical uses claim to have hair restoring properties.

The review of the all plant shows good medicinal value. All the plants provide hair growth activity. Among topical formulation, the gel formulation is more suitable for topical application and produce cooling effects.


Aim & Objective

4. AIM AND OBJECTIVE




PLANT
PROFILES

5. PLANT PROFILE

1.3 *Tridax Procumbence linn*[29]

7.1.1. COMMON NAME

- English : Coat buttons
- Tamil :Vettukaya-thalai
- Telugu : Raavanaasuruditalakaai
- Keralam : Gobbu sanna savanthi

7.1.2. TAXONOMY

KINGDOM : Plantae-plants

DIVISION : Magnoliphyta-flowering plant

CLASS : Magnoliopesida-dicotyledons

ORDER :Asterasles

FAMILY : Asteraceae-Aster family

GENUS : Tridax L.-tridax

SPIECES : *Tridax procumbans lin.*

Fig.5.1 Whole plant of *Tridax procumbens*

7.1.3.GEOGRAPHICAL DISTRIBUTION

Originally from Central America, the plant has become wide spread as a weed in tropical and subtropical regions[30].

7.1.4. HABITAT

Sunny, dry locations, especially sandy and rocky sites like roadsides, railways, dunes, and waste places, at elevations up to 1,000 metres

7.1.5. MORPHOLOY

- **Leaves**

The leaves are green in colour, characteristic odour,3-7cm long ,1-5 cm wide, lanceolate to ovate shape, lamina pinnatisect sometime 3 lobed , short texture, acute apex, irregularly toothed margin, leaf are arranged opposite, the surface of the leaves rough and scabrous and short petiole.

- **Stem**

Green in colour, characteristic odour, acrid taste, 23 -46cm

- **Flower**

Flower are small,long-penduncled heads,rays florets strap shaped, white colour and disc florets yellow coloured ,achenes black, narrowly obconnical 2.0-2.5mm long with feathery papus.

7.1.6. PHYTOCHEMICAL CONSTITUENTS

Leaves contain protein, carbohydrate, β-sitosterol, tannin, fumaric acid,flavonoids.

2.3 **Lagenaria *siceraria***[31]

Botanical name: Lagenaria *siceraria*

Synonym : Bottle guard

Family : Cucurbitaceae

7.2.1. VERNACULAR NAME

COMMONNAME:Bottle gourd

Malayalam: Churakka, Peccura

Tamil: Sorakkai

Telugu: Sorakaya

Hindi: Titalanki

Kannadam: Kadusore

7.2.2. TAXONOMY

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Cucurbitales
Family	: Cucurbitaceae
Genus	: Lagenaria
Species	: *L. siceraria*

Fig. 5.2 *lagenaria siceraria*

7.2.3. HABITAT AND HABITATION[32]

It is obscure, though it is probably Asia or Africa. A plant of the moist, lowland tropics and subtropics .It can be grown at elevations up to 1,600 meters, but economic crops are only produced below 500 meters.

7.2.4. MORPHOLOY[33]

Stems

Prostrate or climbing, angular, ribbed, thick, brittle, softly hairy, up to 5 m long, cut stems exude no sap.

Leaves

Simple, long petioled, 5-lobed, cordate, pubescent, shortly and softly hairy, broadly egg-shaped, kidney-shaped, or heart-shaped in outline, undivided, angular, or faintly 3–7 lobed, lobes rounded, margins shallowly toothed, crushed leaves nonaromatic.

Fruits

Are large, variable, cylindrical, flask-shaped or globose with a constriction above the middle; fleshy, densely hairy, indehiscent, green, maturing yellowish or pale brown, pulp drying out on ripening, leaving a thick, hard, hollow.

Seeds

Many, embedded in a spongy pulp, compressed, with two flat facial ridges, in some variants rather irregular and rugose

7.2.5. PHYTOCHEMICAL CONSTITUENTS

The edible portion of the plant contains proteins, carbohydrates mineral matters calcium, phosphorus other mineral matters iron, sodium, potassium, iodine. Carbohydrates, amino acids, good source of B vitamins and poor source of ascorbic acid

3.3 Trichosanthes cucumarina[34,35]

Botanical name: *Trichosanthes cucumarina*

Synonym : Snake guard

Family : Cucurbitaceae

7.3.1. VERNACULAR NAME

Common name: Khyar, Pudal, Snake Gourd,

Sanskrit &Hindi: Chachinda, Janglipadvel, chachinga.

Marathi : Padwal

Guajarati : Padavali

Telugu :Lingapotla ,Potlakaaya(fruit)

Tamil :Pudalankaai ,Peipudal

Kannada :Padavalakayi

Beng. :Chichinga

Mal. :patavalanga

Punjab :galartori

7.3.2. TAXONOMICAL CLASSIFICATION

KINGDOM : Plantae (plantes, Planta, Vegetal, plants)

DIVISION : Tracheophyta – vascular plants, tracheophytes

CLASS : Magnoliopsida

ORDER : Cucurbitales.

FAMILY : Cucurbitaceae

GENUS : Trichosanthes L.

SPECIES : *Trichosanthes cucumarina var. anguina*

Fig.5.3 *Trichosanthes cucumarina*

The Trichosanthis is wildly distributed around the tropical and subtropical areas ,south and southeast Asia including India, Bangladesh, Nepal, Pakistan, Sri Lanka, Indonesia, Malaysia, Myanmar, Sounthern China.

7.3.3. HABITAT AND HABITATION

The trichosanthis is wildly distributed around the tropical and subtropical areas, south and southeast Asia including India, Bangladesh, Nepal, Pakistan, Sri Lanka, Indonesia, Malaysia, Myanmar, Sounthern China

Warm season crop but depending upon the locality, the vine thrives in rich, loamy soils, other soils with good drainage and rich in organic matter are suitable**.**

7.3.4. MORPHOLOGY

- **Leaf**

The leaves- are 5.8-12.5 cm long of various shapes, lobed, a little broader than long, orbicular reniform or broadly ovate, distantly denticulate, more or less deeply 5(rarely3-7) lobed, the lobes broad, acute, glabrous, less pubescent or when old sometimes scabrid beneath, base deeply cordate, the sinus often sub-rectangular, petioles 2.5-7.5 cm long, striate, pubescent.

- **Stem**

Very long, Slender, furrowed, sub-glabrous, tendrils

- **Flower**

Monoecious, male flowers in axillary racemes, peduncles 5-115 cm long, bearing 8-15 flowers near the apex, female flowers axillar, solitary

- **Fruits**

Sometimes up to 4 meter in length and up to 7.5cm thick, often twisted, green when young, or pale green, white, sometime striped, changing to bright orange when ripe.

7.3.5. PHYTOCHEMICAL CONSTITUENTS[36,35]

Proteins, fat, fiber, other carbohydrates, minerals matter, calcium, oxalic acid, phosphorus, iron, magnesium, sodium, potassium, copper, sulphur, chlorine, thiamine, riboflavin, nicotinic acid, vitamin c, carotene. The ripen fruits contain lycopene and β-carotene it have more antioxidant properties and which rich in fibre content.

Of

6. PLAN OF WORK

- Authentication
- Collection of Plant
- Drying of Plant
- Herbarium of the plant
- Phytochemical work
    - ✓ Physio-Chemical constants analysis
    - ✓ Preliminary chemical test
        - **a. Preliminary phytochemical screening of powders and extracts**
        - **b. Fluorescence analysis of plants powder and extracts**
    - ✓ Extraction
    - ✓ Quantitative analysis
        - ❖ Determination of total flavonoid compounds
        - ❖ Determination of total phenolic compounds
        - ❖ Determination of antioxidant properties
    - ✓ Chromatography
        - ❖ Thin layer chromatography
- Development of gel formulation
    - ✓ Preparation of gel formulation
    - ✓ Standardization of gel formulation
- **PHARMACOLOGICAL STUDIES**
    - ✓ Clinical evaluation of hair growth activity of formulated gel.


METHODOLOGY

7. METHODOLOGY

7.1 LIST OF MATERIALS AND THEIR USES IN FORMULATION

The materials used in the study were as follows:

Table 7.1 List of materials and their uses in formulation

S.no	Name of the Materials	Use in formulation
1.	*Legenaria siceraria* (Leaves)	Active Ingredient
2.	*Trichosanthes cucumerina*(Leaves)	Active Ingredient
3.	*Tridax procumbens* (Leaves)	Active Ingredient
4.	Carbopol 934	Gelling agent
5.	Povidone	Suspending agent
6.	Polyethylene Glycol	Moisturizing agent
7.	Tuber rose oil	Flavoring agent
8.	Demineralized Water	Solvent

7.2 PHYTOCHEMICAL STUDIES

The Phytochemical investigation of a plant involves authentication and extraction of plant material; qualitative and quantitative evaluations; separation and Parallel to this may be the assessment of pharmacological activity.

7.2.1. PHYSIO-CHEMICAL CONSTANTS [37,38,39]

Shade dried powdered plant materials for used for the determination of the physio chemical constants in accordance with the WHO guidelines.

7.2.1.1. DETRMINATION OF ASH VALUES

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining after incarnation is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or edibility added to it, as a form of adulteration.

Ash value of a crude drug is defined as the inorganic residue remaining after

incineration, which complies of inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Hence it is used for the determination of the quality and purity of the crude drug in the powdered form.

❖ TOTAL ASH

Total ash method is designed to measure the total amount of material remaining after ignition. They include both physiological ash which is derived from plant tissue itself and non-physiological ash which is the residue of extraneous matter adhering to the plant surface.

Procedure:

Silica crucible was heated to red hot for 30 minutes and cooled in the desiccators Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tarred silica dish at a temperature not exceeding 450^0C until the sample is free from carbon, cooled in desiccators and weighed. The ash obtained was weighed. The percentage of total ash was calculated.

❖ WATER SOLUBLE ASH:

The difference in weight between the total ash and the residue after treatment of the total ash in water.

Procedure:

Total ash obtained is boiled for 5 minutes with 25 ml of water, insoluble matter were collected in an ashless filter paper, washed with hot water and ignite for 15 min at a temperature not exceeding 450^0. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per gram of air-dried material.

$$\text{Water soluble ash} = \frac{\text{Weight of residue obtained}}{\text{Weight of the sample taken}} \times 100$$

❖ ACID INSOLUBLE ASH:

The residue obtained after boiling the total ash with dilute hydrochloric acid, the remaining insoluble matters are ignited and measured. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure:

To the crucible containing total ash of the sample, 25 ml of dilute hydrochloric acid is added. The insoluble matter is collected on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Filter paper containing the insoluble matter to the original crucible, dry on hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators for 30 minutes and weighed without delay. Content of acid-insoluble ash with reference to the air dried drug is calculated.

$$\text{Acid insoluble ash} = \frac{\text{Weight of the residue obtained}}{} \times 100$$

Weight of the sample taken

❖ **SULPHATED ASH**

Sulphated ash test is used to measure the amount of residual substance not volatilized from a sample. These tests are usually used to determine the content of inorganic substance.

Procedure:

Silica crucible was heated to redness for 10 minutes, allowed to cool in a desiccator and weigh. 2 g of sample were accurately weighed, ignited gently then thoroughly chared. Cool, moistened the residue with 1 ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800 ± 25^{o} until all black particles have disappeared. Crucible are allowed to cool, add few drops of sulphuric acid and heat. Ignite as before, allow to cool and weigh. This process is repeated until two successive weighing differ by more than 0.5 mg.

Sulphated ash = $\frac{\text{weight of the residue obtained}}{\text{Weight of the sample taken}} \times 100$

7.2.1.2. DETERMINATION OF EXTRACTIVE VALUES:

Extractive values are useful for the evaluation of phytoconstituents especially when the constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in a crude drug.

❖ **Determination of water soluble extractive**

5gm of air dried coarsely powdered sample was weighed and macerated with 100ml of chloroform water (95ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for rest eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and was dried at 105°C for 1 hour in the hot air oven and cooled in desiccators for 30min and weighed. The process was repeated till a constant weight was obtained; the percentage of water soluble extractive value was calculated with reference to the air dried drug.

Water soluble extractive value = $\frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$

❖ **Determination of alcohol soluble extractive**

5gm of the coarsely powdered sample was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105°C for 1hour in a hot air oven. The dish was cooled in desiccator and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to

the air dried drug was calculated.

Alcohol soluble extractive = $\frac{\text{weight of the dried extract}}{\text{weight of the sample taken}} \times 100$

❖ **Determination of ether soluble extractive**

The type of ether soluble extractive values determined for evaluation of crud drug are volatile and non-volatile ether soluble extractives. The volatile ether soluble represent volatile oilcontent of drug, while non-volatile ether soluble extractives represent resin, fixed oils or colouring matter present in drugs. The percentage of ether soluble extractive was calculated

Ether soluble extractive = $\frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$

7.2.1.3. DETERMINATION OF MOISTURE CONTENT:

❖ **LOSS ON DRYING**

10 g of the sample substances (without preliminary drying) was taken in a tarred evaporating dish. Use of high speed mill in preparing the samples are avoided. The sample in the tarred evaporating dish were placed in the drying chamber (105°C) for 5 hours and weigh. Drying and weighing is continued every one hour interval until the difference between the two successive weight is not more than 0.25 percent. Constant weight is reached when the two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.001 g difference. Percentage moisture content is compared with respect to the air dried sample.

% Moisture content = $\frac{\text{Final weight of the sample}}{\text{Initial weight of the sample}} \times 100$

7.2.2. PREPARATION OF EXTRACT [40]

Extraction is the preliminary step involved in the phytochemical studies. Based on solvent's polarity metabolites are extracted and according to the solubility of the constituents in the solvent. The method of extraction is hot percolation method.

❖ **HOT PERCOLATION METHOD**

About 200g of coarsely powdered plant was extracted with solvents of increasing polarity like Hexane, Chloroform, Ethyl acetate and Ethanol at 60-70°C. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, color and consistency of all the extracts were noted and were taken up for further

detailed phytochemical and pharmacological screening.

7.2.3. QUALITATIVE PHYTOCHEMICAL ANALYSIS [41,42]

Qualitative analysis for various phytoconstituents in the dried powders and extracts all the raw materials were carried out using different reagents are mentioned below.

❖ DETECTION OF ALKALOIDS

Dragendroff's Test

The powder/extract was dissolved in 5ml of distilled water, to this 5ml of 2M Hcl was added. Then 1ml of Dragendroff's reagent was added and examined for an immediate formation of an orange red precipitate.

Mayer's Test

The powder/extract was mixed with little amount of dilute Hcl and Mayer's reagent and examined for the formation of white precipitate.

Wagner's Test

The powder/extract was mixed with Wagner's reagent and examined for the formation of reddish brown precipitate.

❖ DETECTION OF GLYCOSIDES

Bontrager's Test

The powdered plant material/extract was boiled with 1ml of sulphuric acid in a test tube for few minutes. The solution was filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of the solvent was separated and shaken with half of its volume of dilute Ammonia. Formation of a rose pink to red colour in the ammoniacal layer indicates the presence of glycosides.

Modified Bontrager's Test

The test material was boiled with 2ml of the dilute sulphuric acid. This was treated with 2ml of 5% freshly prepared aqueous ferric chloride solution for 5 min and

shaken with equal volume of chloroform .the lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. Formation of a rose pink to red colour in the ammoniacal layer indicates the presence of glycosides.

Legal's Test

The test material when treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of a pink to blood red colour indicates the presence of cardiac glycosides.

❖ DETECTION OF STEROIDS

Liebermann-Buchards Test

The powdered drug/extract was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the sides of the test tube. If a brown ring is formed at the junction of two layers and upper layer turns green, it shows presence of steroids.

Shinoda Test

To the solution of extract few piece of magnesium turnings and drops of concentrated Hcl were added. If a pink crimson red or occasionally green to blue colour appears after few minutes, this indicates the presence of flavonoids.

Alkaline reagent Test

To the test solution, few drops of sodium hydroxide solution were added. If there is a formation of intense yellow colour which turns to colourless on addition of few drops of dilute acid, this indicates presence of flavonoids.

❖ DETECTION OF CARBOHYDRATES

Molisch's Test

To the test solution, few drops of alcoholic alpha-napthol and few drops of concentrated Sulphuric acid were added through the sides of the test tube. Appearance

of purple to violet colour ring at the junction indicates the presence of carbohydrates.

Fehling's Test

The test solution was mixed with Fehling's I and II, heated and examined for the presence of red colouration for the presence of sugar.

❖ DETECTION OF PHENOL

Ferric chloride Test

A small quantity of powdered drug/ extract was dissolved in 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for appearance of blue or green colour.

❖ DETECTION OF PROTIENS

Biuret Test

The sample was treated with 5-8 drops of 1% w/w copper sulphate solution and 1ml of 5% sodium hydroxide. If a violet colour is formed it indicates the presence of proteins.

❖ DETECTION OF TANNINS

Lead acetate Test

The test solution was mixed with basic lead acetate solution and examined for formation of white precipitate.

Ferric chloride Test

A few drops of 5% aqueous ferric chloride solution was added to 2 ml of aqueous extract of the drug and examined for the presence of bluish black colour.

❖ DETECTION OF SAPONINS

A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3minutes. Development of any honey comb

like froth was examined.

❖ DETECTION OF GUMS AND MUCILAGE

A small quantity of powdered drug/extract was dissolved in 5 to 10 ml of acetic anhydride by means of heat, cooled and 0.05ml of concentrated Sulphuric acid was added. Formation of bright purplish red colour indicates the presence of gums and mucilage.

❖ DETECTION OF FIXED OILS AND FATS

A small quantity of extract was pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils and fats.

7.2.4. FLUORESCENCE ANALYSIS [43]

Fluorescence analysis was carried out in day light and in UV light. The powdered plant raw materials and their extracts were treated with various reagents and solvents to identify the presence of chromophores. The fluorescence was observed in day light and in short and long UV light 254nm and 365nm respectively.

7.2.5. QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENT

❖ DETERMINATION OF TOTAL FAVONOIDS[44]

METHODS

Ultra Violet spectroscopy method was carried out for the determination of total flavonoids content.

Preparation of Standard Stock solution

Accurately weighed 25 mg of Quercetin standard transferred to 100 ml of volumetric flask and dissolved with dimethyl sulfoxide (DMSO). The serial dilution (20mcg, 40mcg, 60mcg, 80 mcg, 100 mcg) were made with dimethyl sulfoxide.

Preparation of Test Solution

The leaf extract was weighed accurately equal to the weight of Standard Quercetin and transferred to 100 ml volumetric flask and the extract dissolved with dimethyl sulfoxide (DMSO). The dilution was made with dimethyl sulfoxide.

Procedure

From the prepared solution of standard and test solutions 2ml was withdrawn from each concentration to the test tube and added equal volume of 2% Aluminium Chloride solution to every single concentration. Incubate the solution about 10 minute at ambient temperature. After 10 minute, Standard and sample solution measure the absorbance of spectrophotometrically at 435 nm with the standard and test sample solutions.

❖ DETERMINATION OF TOTAL PHENOLIC CONTENT

(Folin – Ciocalteu's assay)

- **METHODS**

  Ultra Violet spectroscopy method was carried out for the determination of total flavonoids content

- **PREPARATION OF 16 %Na_2Co_3**

  16gm w/w of sodium carbonate dissolved in 100ml of water.

- **PREPARATION OF STANDARD**

  40mg of Gallic acid standard transferred 100ml of standard flask, Add to this 50 ml distilled water and shake well until the sample dissolved. Made volume with distilled water and withdrawn 10ml of the above solution into 100ml standard flask then make up to volume with distilled water.

- **PREPARATION OF TEST SOLUTION**

The 1gm of extract transferred to 250ml of flask, Add to this 50ml of water and heated on a water bath about 45 minutes. Then the solution was cool and made up to volume with distilled water, the solution was centrifuged at 5000rpm about 20 minutes. Then 2ml of superatted liquid transferred to 25ml of flask then made volume with water

- **BLANK** Purified water

- **PROCEDURE**
  - Pipetted out 2ml of Standard/test/blank to 25 ml of standard flask.
  - To the above solution add 1 ml of folin-ciocalteu's reagent and made upto volume with saturated 16% of Sodium Carbonate solution.
  - Kept the solution aside about 30 minutes.
  - Absorbance of the solution was measured against blank by Spectrophotometrically at 760nm

7.3. DEVELOPMENT AND STANDARDISATION OF POLY HERBAL FORMULATION [46,47,48,49]

The prepared extracts were taken for the preparation of topical gel with water soluble gelling agent Carbopol 934, Propylene glycol 400, Povidone, Glycerin, Distilled water.

Method for Preparation of Gel Containing Extracts (1% w/w)

1 g of Carbopol 934 was dispersed in 50 ml of hot distilled water with continuous stirring. 5ml of distilled water was taken and required quantity of povidone was dissolved. Cool the solution, then to that added Propylene glycol 400. Further required quantity of prepared leaves extracts were mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and tri-ethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

Table 7.2 Formula for gel preparation

Ingredients	**Quantity Specified**
Carbopol-934	1 g
Propylene glycol-400	Quantity sufficients
Povidone	5%
Triethanolamine	1.2ml(quantity sufficient)
Distilled water	Make up to 100

Standardisation of Topical Gel Formulation

- **Physical Evaluation**

  Physical parameter(50)s such as colour and appearance were checked.

- **Measurement of pH**

  pH of the gel was measured by using pH meter.

- **Spreadability**

  Spreadability of the prepared gel was determined by wooden block and glass slide apparatus.

Apparatus consists of a wooden block, which was provided by a pulley at one end. Spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on this ground slide. The gel was then placed between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 kg weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80gms. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability.

Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where, S = Spreadability,

M = Weight in the pan (tied to the upper slide),

L = Length moved by the glass slide and

T = Time (in sec.) taken to separate the slide completely each other.

Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle.

Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Skin irritation test

Test for irritation was performed on human volunteers. The prepared gel was applied on area of 2 square inch to the back of hand to volunteers who are selected for the evaluation of hair growth gel. The volunteers were observed for lesions or irritation.

7.4. CLINICAL EVALUATION OF HAIR GROWTH [51],

The permission for the clinical study was granted by the Institutional ethical committee. The committee approved the proposal and granted the,

Certificate NO. 3112206, Dated 14.12.2016

The prepared and standardized gel formulation was evaluated clinically. The clinical evaluation carried out in the Department of Dermatology, Rajivgandhi Government General Hospital, Chennai.

Selection of human volunteers

Inclusion Criteria

- Both female and male, whose age is more than 18 years
- Patients, who are willing to give written, signed and dated informed consent to participate in the study.
- Patients who are clinically diagnosed for diffuse hair loss are included in this study.

Exclusion Criteria

- Pregnant or breast feeding or planning to become pregnant during the study period.
- Known case of hypersensitivity investigational drug content.
- Patients using any other treatment for this indication at the same time.
- Received any other investigational medicinal therapy within 30 days and topical therapy within 7 days prior to screening.
- Patients suffering from any active general illness or have history of any general illness.

After the Selection of human volunteer, the nature of study was explained and they were request to read and sing both the informed consent form and information sheet. The consent form and information designed as follows

INFORMED CONSENT FORM

Name of the Participant:

Name of the Principal (Investigator):

Name of the Institution:

Documentation of the informed consent

- I ---------- have read it has been for me, the information in this form. I was free to ask any questions and they have been answered. I am over 18 years of age and exercising my free power of choice, here by give my consent as a participant in the study.
- I have read and understood this consent form and the information provided to me.
- I have had the consent document explained to me.
- I have been explained about the nature of the study.
- I have been explained about the rights and responsibilities by the investigator.
- I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
- I hereby give permission to the investigators to release the information obtained from me as a result of participation in this study to the regulatory authorities, government agencies and Institutional ethics Committee. I understand that they are publicly presented.
- I have understood that my identity will be kept confidential if any data are publicly presented.
- I have had my questions answered to my satisfaction.
- I have decided to be in the research study.
- I am aware that if I have any question during this study, I should contact at one of the addresses listed above. By signing this consent for I attest that the information given in this document has been clearly explained to me and apparently understood by me. I will be given a copy of this consent document.

INFORMATION SHEET

Investigator:

Name of the Participant:

- You are invited to take part in this study. The information in this document is meant to help you decided whether or not to take part. Please feel free to ask if you have any queries or concerns
- We are conducting a study on "**DEVELOPMENT AND STANDARDIZATION OF POLY HERBAL GEL FORMULATION AND ITS CLINICAL STUDIES**" among the patients attending Rajive Gandhi Government Hospital, Chennai
- And for that your participation may be valuable us.
- The purpose of the study is to determine the hair growth stimulation of the poly herbal gel formulation and to determine the effectiveness of the poly herbal gel formulation.
- The patient will be subjected to determine the hair growth efficiency before and after application of the herbal gel and the measurement of hair growth at interval of 7 days initially and later it will extended to 15 days.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The result of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Study groups and duration of study

30 volunteers selected according to the inclusion criteria. They are to be divided into 2 groups control and test. The control group receives gel without extract medication. The test group receives the formulated herbal gel. Duration of the study is 90days.

Pre evaluation parameter of volunteers

- Measurement of hair texture, examined by simple method of touching the patient's hair,
- Hair density/cm.sq area :examined at a fixed site of scalp with Trichoscope
- Hair loss: counted after constant combing of patient's hair for one minute with the comb

Application of gel

The formulated gel is applied over the scalp during the course of study. If there is any sign of irritation or discomfort to the patient, the gel is discontinued.

Evaluation parameters for hair growth (During the period of study)

- Measurement of hair texture, examined by simple method of touching the patient's hair,
- Hair density/cm.sq area :examined at a fixed site of scalp with Trichoscope
- Hair loss: counted after constant combing of patient's hair for one minute with the comb

RESULT AND DISCUSSION

8. RESULTS AND DISCUSSION

8.1.PHYSIO-CHEMICAL CONSTANTS.

8.1.1. ASH VALUE

Physiochemical parameters are mainly used in the assessment of purity and quality of the powdered drug. Ash values of a drug give an idea of the earthy matter or inorganic elements and other impurities present along with the drug.

Ash values are mainly used in judging the purity and quality of the drug is the indicative of contamination, substitution and adulteration. The total ash usually consists of carbonate, phosphate and silicates. Total ash, acid insoluble ash and water soluble ash were done and reported in the table below.

- **Total ash**

The total ash content of the raw materials were determined, taking sample from the Collected materials.(Table 8.1)

Table 8.1 Total Ash Value

S.NO	Ingredient	Total ash (%w/w)	*Limits (%w/w)
1.	*Legenaria siceraria* (Leaves)	9.3±1.8	Not more than 12
2.	*Trichosanthes cucumerina* (Leaves)	8.6±1.2	Not more than 15
3.	*Tridax procumbens* (Leaves)	10.6±0.9	Not more than12

Values are expressed as Mean ± SD, n=3

- **Acid insoluble Ash**

From the total ash, the acid insoluble ash content of the individual raw materials were determined and results enumerated.(Table 8.2)

Table 8.2 Acid Insoluble ash Value

S.NO	Ingredient	Acid insoluble ash (%w/w)	*Limits (%w/w)
1.	*Legenaria siceraria* (Leaves)	0.5±1.2	Not more than 0.6
2.	*Trichosanthes cucumerina*(Leaves)	1.4±1.5	Not more than2.5
3.	*Tridax procumbens* (Leaves)	3.01±2.5	Not more than 3.5

Values are expressed as Mean ± SD, n=3

❖ **Sulphated Ash**

Sulphated content of raw materials was determined, the values obtained and their acceptable limits defined are given.(table.8.3)

Table 8.3 Sulphates Ash

S.NO	Ingredient	Sulphated ash (%w/w)	*Limits (%w/w)
1.	*Legenaria siceraria* (Leaves)	8.2±1.3	Not more than10
2.	*Trichosanthes cucumerina*(Leaves)	7.3±1.7	Not more than 8
3.	*Tridax procumbens* (Leaves)	11.6±2.1	Not more than 20

Values are expressed as Mean ± SD, n=3

8.1.2. DETERMINATION OF EXTRACTIVE VALUE

❖ Water Soluble Extractive

Water soluble extractive values for the raw materials in water were determined and the results were given.(Table 8.4)

Table 8.4 Water soluble extractive value for Raw materials

S.NO	Ingredient	Water Extractive value (%w/w)	*Limits (%w/w)
1.	*Legenaria siceraria* (Leaves)	26.3±16	Not less than 25
2.	*Trichosanthes cucumerina* (Leaves)	20.6±1.3	Not less than18
3.	*Tridax procumbens* (Leaves)	21.4±2.1	Not less than 20

Values are expressed as Mean ± SD, n=3

❖ Alcohol Soluble Extractive

Alcohol soluble extractive values for the raw materials in ethanol 95% were determined and the results were given in table

Table 8.5 Alcohol soluble extractive value for Raw materials

S.NO	Ingredient	Alcohol Extractive value (%w/w)	*Limits (%w/w)
1.	*Legenaria siceraria* (Leaves)	16.3±1.2	Not less than 10
2.	*Trichosanthes cucumerina* (Leaves)	11.2±1.4	Not less than 8
3.	*Tridax procumbens* (Leaves)	12.6±0.9	Not less than 12

Values are expressed as Mean ± SD, n=3

❖ **Ether Soluble Extractive**

Ether soluble extractive values for the raw materials in ether were determined and the results were given in table

Table 8.6 Ether soluble extractive value for Raw materials

S.NO	Ingredient	Ether soluble Extractive value (%w/w)	*Limits (%w/w)
1.	*Legenaria siceraria* (Leaves)	2.1±0.8	Not less than 1
2.	*Trichosanthes cucumerina* (Leaves)	1.9±1.2	Not less than 1.5
3.	*Tridax procumbens* (Leaves)	2.7±1.6	Not less than 2

Values are expressed as Mean ± SD, n=3

8.1.3. MOISTURE CONTENT

Loss on Drying

Loss on dry analysis in the raw materials were carried out and the results were recorded and results in Table 8.7

Table 8.7 Loss on Drying

S.NO	Ingredient	LOD (%w/w)	*Limits (%w/w)
1.	*Legenaria siceraria* (Leaves)	4.8	Not more than15
2.	*Trichosanthes cucumerina* (Leaves)	6.3	Not more than 15
3.	*Tridax procumbens* (Leaves)	8.4	Not more than 15

8.2. PREPARATION OF EXTRACTS

The shade dried all plants materials raw were extracted in soxhlet extractor with the universal solvent ethanol. All the extracts were concentrated using rotary vacuum evaporator. The percentage yield was calculated for every extract in terms of dried weight of plant materials. The colour and consistency of the concentrated extracts are given in table no.2

Table 8.8 Percentage yield of extracts

S.NO	PLANT NAME	SOLVENT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD (%W/W)
1.	*Legenaria siceraria* (Leaves)	Ethanol (95%)	Continuous Hot percolation method using Soxhlet apparatus	Semisolid	Dark green	5.58
2.	*Trichosanthes cucumerina* (Leaves)			Semisolid	Dark green	4.76
3.	*Tridax procumbens* (Leaves)			Semisolid	Dark green	6.46

8.3. QUALITATIVE ESTIMATION OF PHYTOCONSTITUENTS

The raw material powders and all the extracts were subjected to qualitative phytochemical analysis to identify the various phytoconstituents present in it, as per the standard procedures. The results are given in the Table 8.8

Table 8.8 Preliminary phytochemical analysis of powder and extracts of raw materials

Chemical constituents	***Lagenarria siceraria***		***Trichosanthes cucumerina***		***Tridax procumbens***	
	Powder	**Extract**	**Powder**	**Extract**	**Powder**	**Extract**
Steroids	+	+	+	+	-	-
Glycosides	-	-	-	-	+	+
Saponins	-	-	-	-	-	
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Proteins	+	+	+	+	-	-
Alkaloids	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-
Fats and oils	-	-	+	+	-	-

+ indicates presence , - indicates absence

8.4. FLUORESCENCE ANALYSIS

Fluorescence analysis for the extracts and the powdered drug were carried out with various reagents to identify the presence of chromophores. The importance of fluorescence analysis is that UV light shows the fluorescent nature of the compound whereas fluorescence cannot be observed in day light. Hence it is performed according to the standard procedures. The results are shown in Table 8.9

Table 8.9 Fluorescence analysis of *Lagenaria siceraria*

TREATMENT	DAY LIGHT	SHORT UV (254 nm)	LONG UV (365 nm)
Drug	Green	Green	Dark green
Saturated picric acid	Yellowish green	Dark green	Yellowish-black
Nitric acid	Radish- brown	Light brown	Brown
Hydrochloric acid	Green	Block	Block
Sulphuric acid (80%)	Block	Block	Block
Glacial acetic acid	Dark green	Green	Block
Iodine solution (N/20)	Dark green	Dark green	Dark Brown
Ferric solution (5% W/V aq. Solution)	Yellowish brown	Dark Brown	Dark Brown

Table 8.10: Fluorescence analysis of *Ttichosanthes cucumerina*

TREATMENT	DAY LIGHT	SHORT UV (254 nm)	LONG UV (365 nm)
Drug	Green	Green	Dark green
Saturated picric acid	Yellowish green	Dark green	Yellowish-black
Nitric acid	Radish- brown	Light brown	Brown
Hydrochloric acid	Green	Block	Block
Sulphuric acid (80%)	Block	Block	Block
Glacial acetic acid	Dark green	Green	Block
Iodine solution (N/20)	Dark green	Dark green	Brown
Ferric solution (5% W/V aq. Solution)	Yellowish brown	Dark Brown	Dark Brown

Table 8.11: Fluorescence analysis of *Ttridax procumbens*

TREATMENT	DAY LIGHT	SHORT UV (254 nm)	LONG UV (365 nm)
Drug	Green	Green	Dark green
Saturated picric acid	Yellowish green	Dark green	Yellowish-black
Nitric acid	Radish- brown	Light brown	Brown
Hydrochloric acid	Green	Block	Block
Sulphuric acid (80%)	Block	Block	Block
Glacial acetic acid	Dark green	Green	Block
Iodine solution (N/20)	Dark green	Dark green	Brown
Ferric solution (5% W/V aq. Solution)	Yellowish brown	Dark Brown	Dark Brown

No fluorescence was observed for the powder as well as extracts indicating the absence of chromophore in the plant.

8.5. QUANTITATIVE ESTIMATION

❖ TOTAL FLAVONOID CONTENT

The determination of total flavonoids from various extracts of *Lagenaria siceraria*, *Trichosanthes cucumerina*, *tridax procumbens* were performed with the Quercetin standard. The accuracy of test made by the serial dilution of Standard and the absorbance was measured spectrophotometrically at 435 nm (Table8.12). The obtained data were plotted as a Standard Calibration curve (Fig.1).

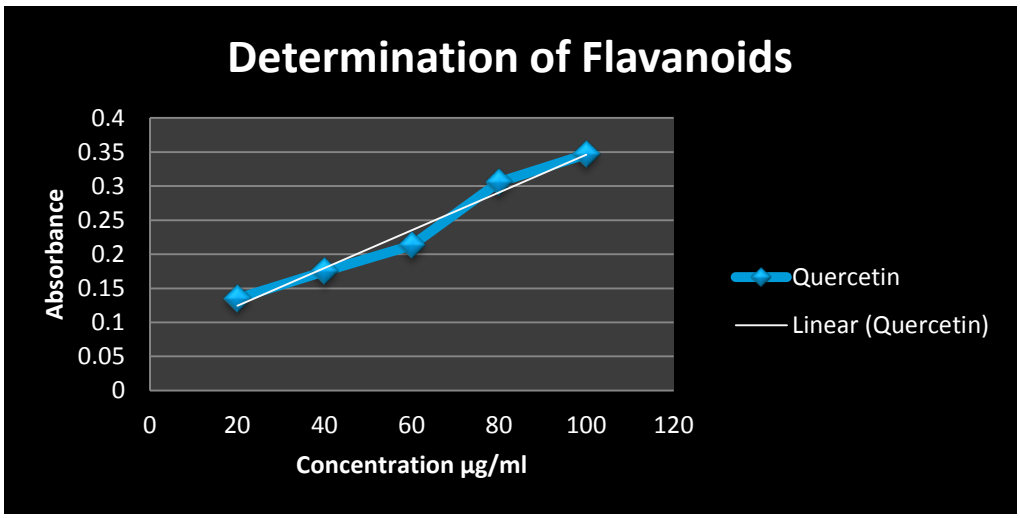
Standard Calibration Curve for Quercetin

To determine the accuracy of the flavonoid compound by plotted the standard absorbance obtained from spectrophotometrically. The calibration curve done by made serial dilution (20mcg, 40mcg, 60mcg, 80 mcg, 100 mcg) of quercetin Standard stock solution, the absorbance plotted against concentration

Table 8.12: Spectrophotometric absorbance of Standard and Sample

S.No	Concentration of standard solution(µg/ml)	Absorbance(435nm)
1.	20	0.135
2.	40	0.175
3.	60	0.213
4.	80	0.306
5.	100	0.347
6.	*Lagenaria siceraria*	0.178
7.	*Trichosanthis cucumarina*	0.165
8.	*Tridax procumbens*	0.159

Figure 8.1: Standard Calibration Curve for Quercetin



From the replicate absorbance value obtained by the spectrophotometry, the calculation of concentration of flavonoid present in 1gm of the extract was calculated by applying the dilution factor. The concentration of each extract obtained (Table 8.13)

Table 8.13 Percentage yield of total flavonoid

S.NO	Sample	Concentration Obtained (mg/gm)	Percentage of Flavonoids Present
1.	*Lagenaria siceraria*	43.43	4.3
2.	*Trichosanthis cucumarina*	39.85	4
3.	*Tridax procumbens*	33.72	3.4

❖ **TOTAL PHENOLIC CONTENT**

Total phenolic content of the individual extract was determined and compared with that of standard. It is shown in table 8.14

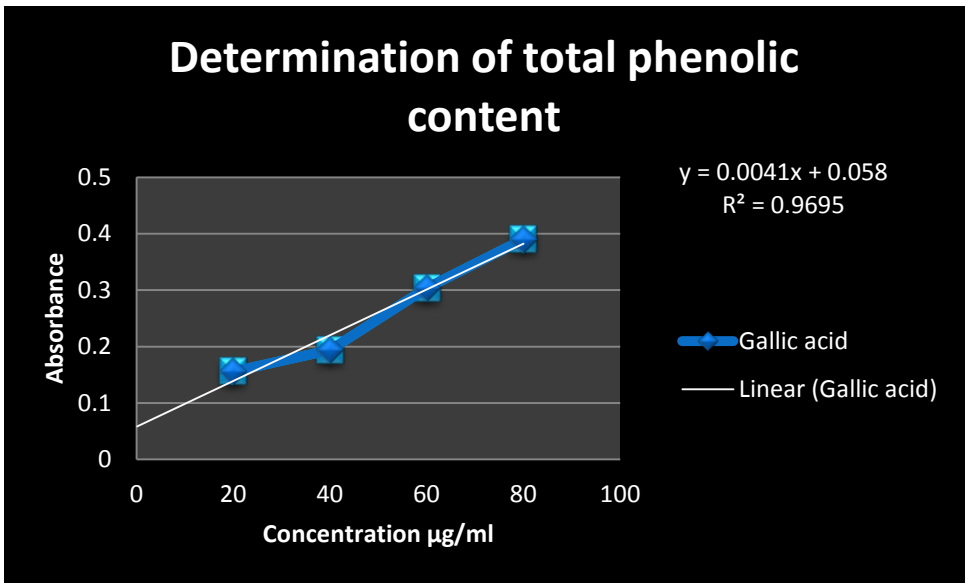
Table 8.14 Spectrophotometric absorbance of Standard and Sample

S.No	Concentration of Standard Solution(µg/ml)	Absorbance(435nm)
1.	20	0.156
2.	40	0.194
3.	60	0.304
4.	80	0.390
5.	*Lagenaria siceraria*	0.266
6.	*Trichosanthis cucumarina*	0.231
7.	*Tridax procumbens*	0.216

Standard Calibration Curve for Gallic acid

To determine the accuracy of the phenolic compound by plotted the standard absorbance obtained from spectrophotometrically. The calibration curve done by made serial dilution (20mcg, 40mcg, 60mcg, 80mcg) of Gallic acid Standard stock solution, the absorbance plotted against concentration

Figure 8.2: Standard Calibration Curve for Gallic acid



From the replicate absorbance value obtained by the spectrophotometry, the calculation of concentration of phenolic content present in 1gm of the extract was calculated by applying the dilution factor. The concentration of each extract obtained (Table 8.15)

Table 8.15 Percentage of phenolic content present in each extract

S.NO	Sample	Concentration Obtained (mg/gm)	Percentage of phenolic content Present(%w/w)
1.	*Lagenaria siceraria*	204.33	20.4
2.	*Trichosanthis cucumarina*	168.27	16.8
3.	*Tridax procumbens*	108.17	10.8

Table 8.16 STANDARDIZATION OF PREPARED POLY HERBAL GEL

S. No.	Parameters	Results
1.	**Physical appearance**	Light green
2.	**pH**	7.1
3.	**Spreadability**	Good
4.	**Viscosity**	43560 cps
5.	**Homogeneity**	Excellent
6.	**Skin irritation test**	No irritation

CLINICAL EVALUATION OF HAIR GROWTH

The prepared and standardized ploy herbal gel formulation was tested on human volunteers. Before the clinical evaluation was carried out ethical clearance was approved by Institutional Ethical Committee. The hair length and diameter was measured by trichoscope and the results were tabulated.

Every testing interval the patients scalp photos taken with the trichoscope (Fig8.3, Fig.8.4).The number of hair present in scalp was counter as per square area.

The obtained mean value from hair count in 1 cm square area for test group was plotted on graph

Figure 8.3 Hair in Scalp

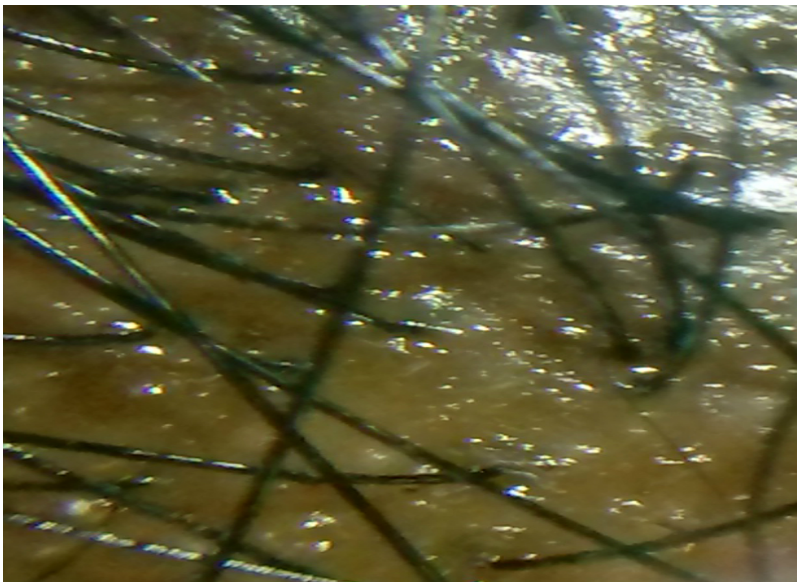


Figure 8.4 Hair in Scalp

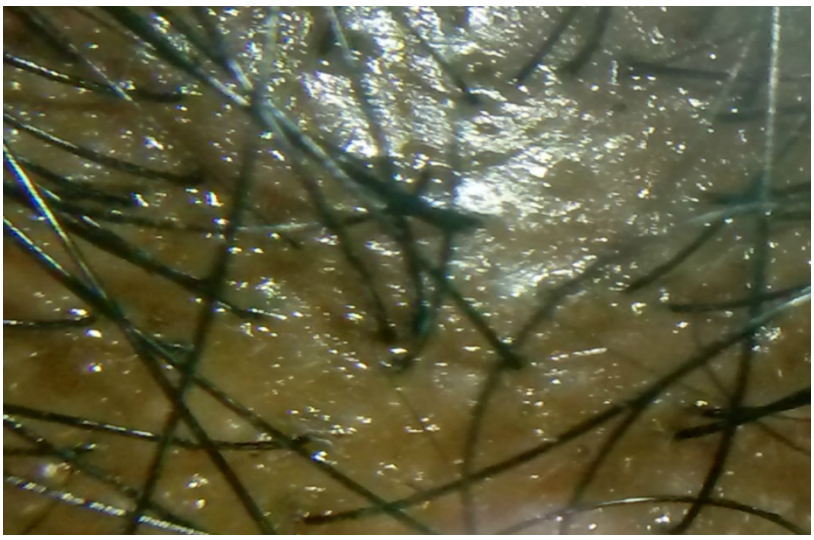
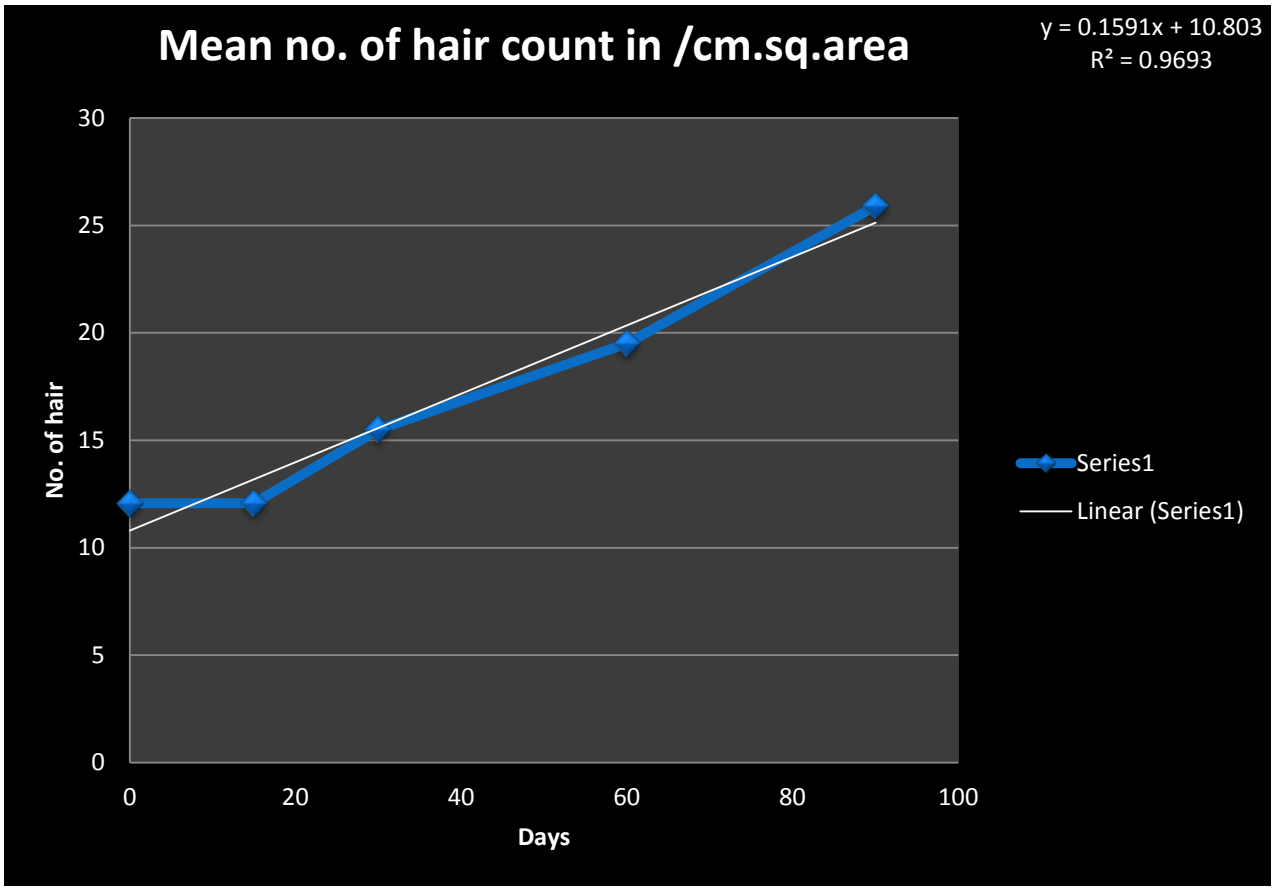


Table 8.17 Hair count in per square area in test group

No. of volunteers	Initial	After 15 days	After 30 days	After 60 days	After 90 days
1.	15	15	20	26	37
2.	10	10	15	20	27
3.	13	12	16	18	29
4.	9	10	12	18	25
5.	15	16	19	22	30
6.	12	10	15	18	22
7.	9	9	14	19	24
8.	15	13	16	24	33
9.	10	9	12	20	25
10.	8	8	11	15	21
11.	10	11	14	19	19
12.	14	15	17	17	20
13.	12	13	16	20	29
14.	16	17	18	20	23
15.	13	13	17	17	24
Mean	**12.07±2.6**	**12.07±2.8**	**15.5±2.6**	**19.5±2.8**	**25.9±5.0**

Fig.8.5 Mean No. of Hair count

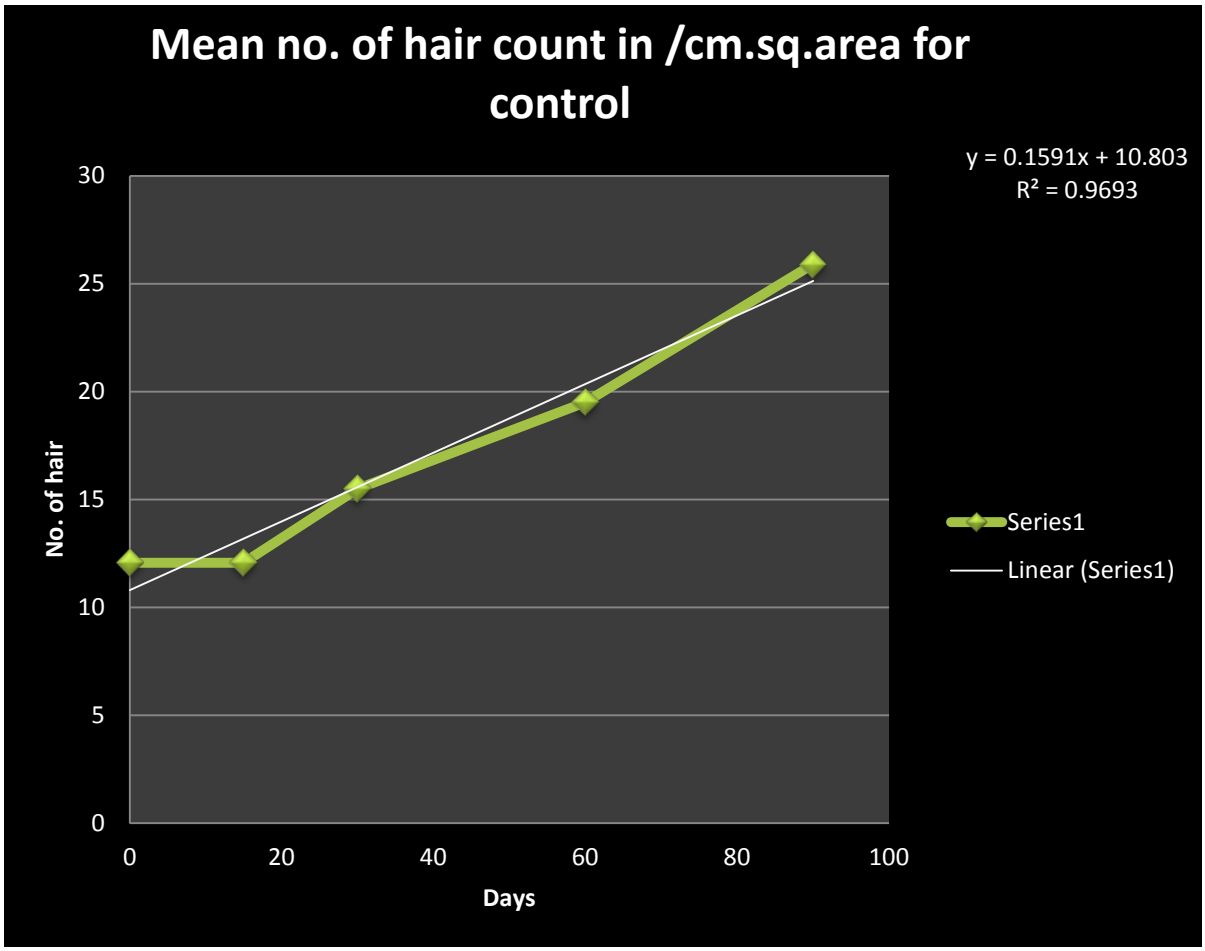


Mean No. of hair count in/cm. sq. area for Test group

Table 8.18 Hair count in per square area in control group

No. of volunteers	Initial 0 days	After 15 days	After 30 days	After 60 days	After 90 days
1.	10	10	12	14	13
2.	14	13	13	15	15
3.	10	11	11	12	12
4.	7	7	8	7	7
5.	15	14	17	16	16
6.	13	11	11	13	14
7.	11	11	12	14	14
8.	12	12	14	15	15
9.	14	13	14	14	16
10.	8	8	10	10	11
11.	12	12	11	13	13
12.	11	11	14	15	14
13.	12	14	14	15	15
14.	9	10	9	11	11
15.	13	13	13	14	14
Mean value	**11.4±2.3**	**11.3±2.02**	**12.2±2.3**	**13.2±2.4**	**13.3±2.35**

Fig 8.6 Mean No. of hair count For Control Group



The hair growth activity of the prepared gel formulation was tested on human volunteers and growth activity was compared with the control group. The obtained mean value (Table 8.18) from both test and standard group were compared Fig

Table 8.18 Meant value of hair count

No. of Days	Test Group	Control Group
0	12.07±2.6	11.4±2.3
15	12.07±2.8	11.3±2.03
30	15.5±2.6	12.2±2.3
60	19.5±2.8	13.2±2.4
90	25.9±5.0	13.3±2.4

Values are expressed as Mean ± SD, n=15

Fig. 8.7Comparison of hair growth of Test with Control

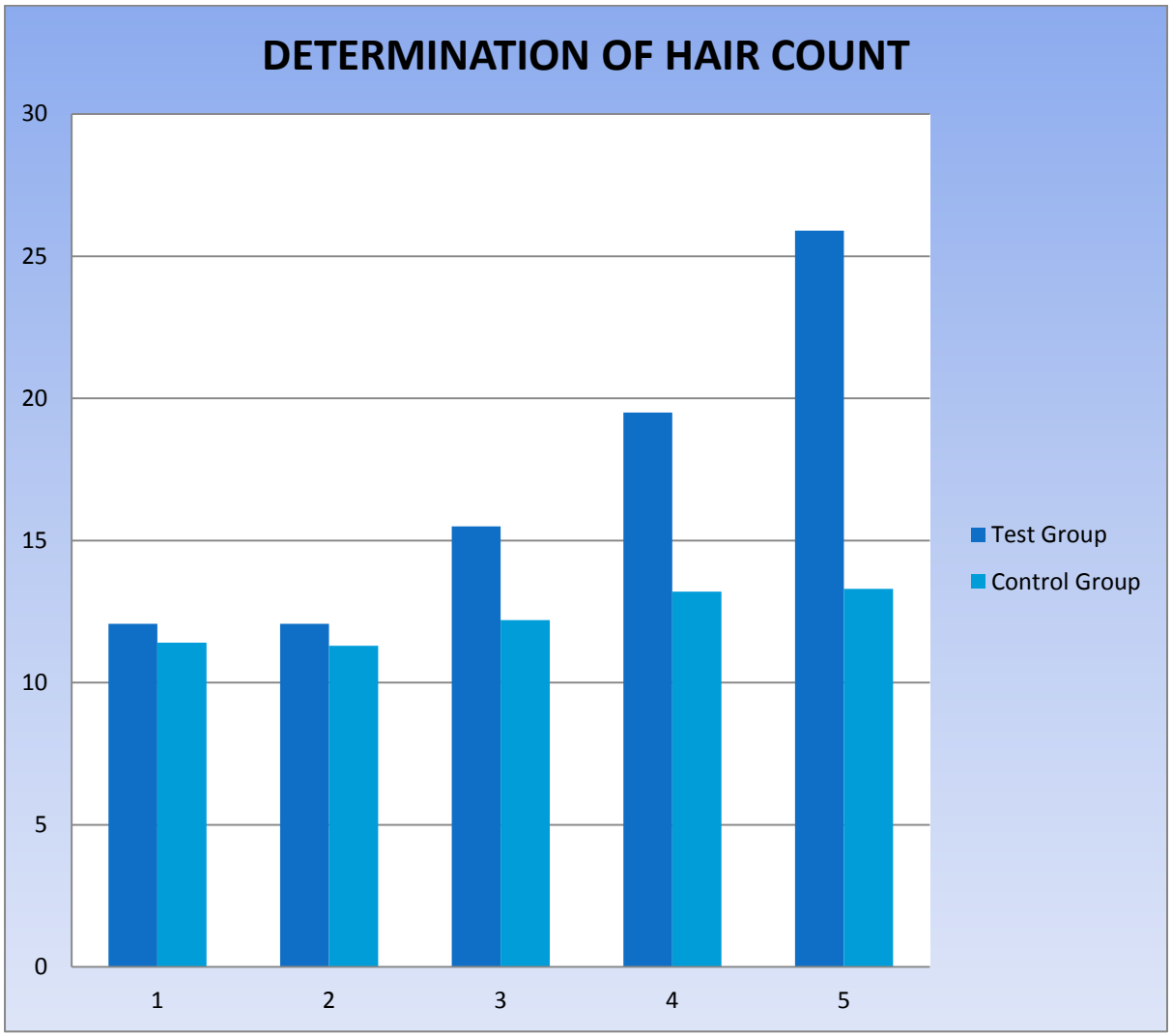


Table 8.19 Mean number of hair loss in Combing test

Parameter	Group	Initial 0 days	After 15 days	After 30 days	After 60 days	After 90 days
Mean hair loss (/combing)	Test	114.5± 6.4	80.4± 12.4	78.2± 11.4	69.3± 10.4	51.5± 15.4
	Control	113.2± 6.4	109.2± 6.4	103.4± 6.7	99.9± 7.9	95.2± 6.8
Perceptible Reduction in hairloss by Patients(%)	Test	-	34.1%	38.6%	45.2%	63.1%
	Control	-	4.0%	10.4%	13.7%	18.0%

Fig. 8.8 Determination of Reduction of Hairloss

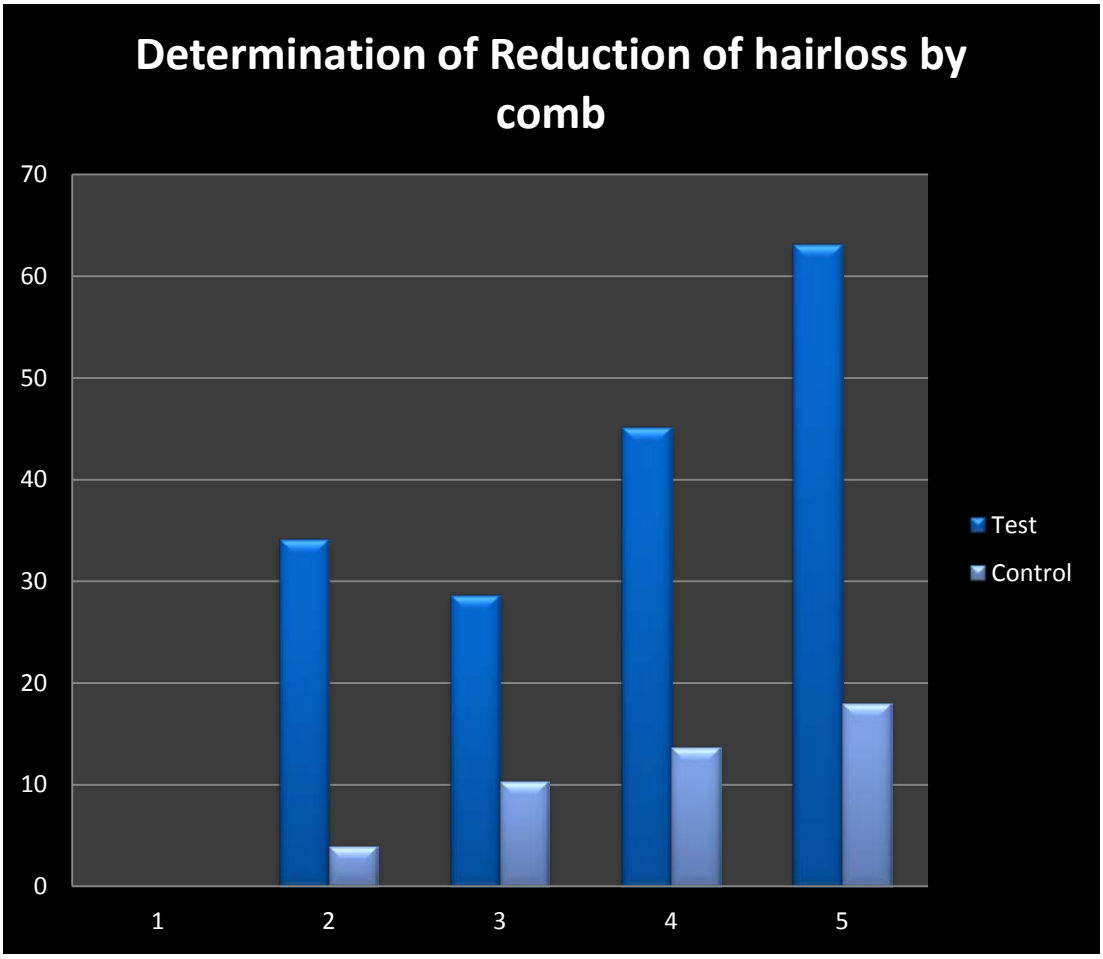
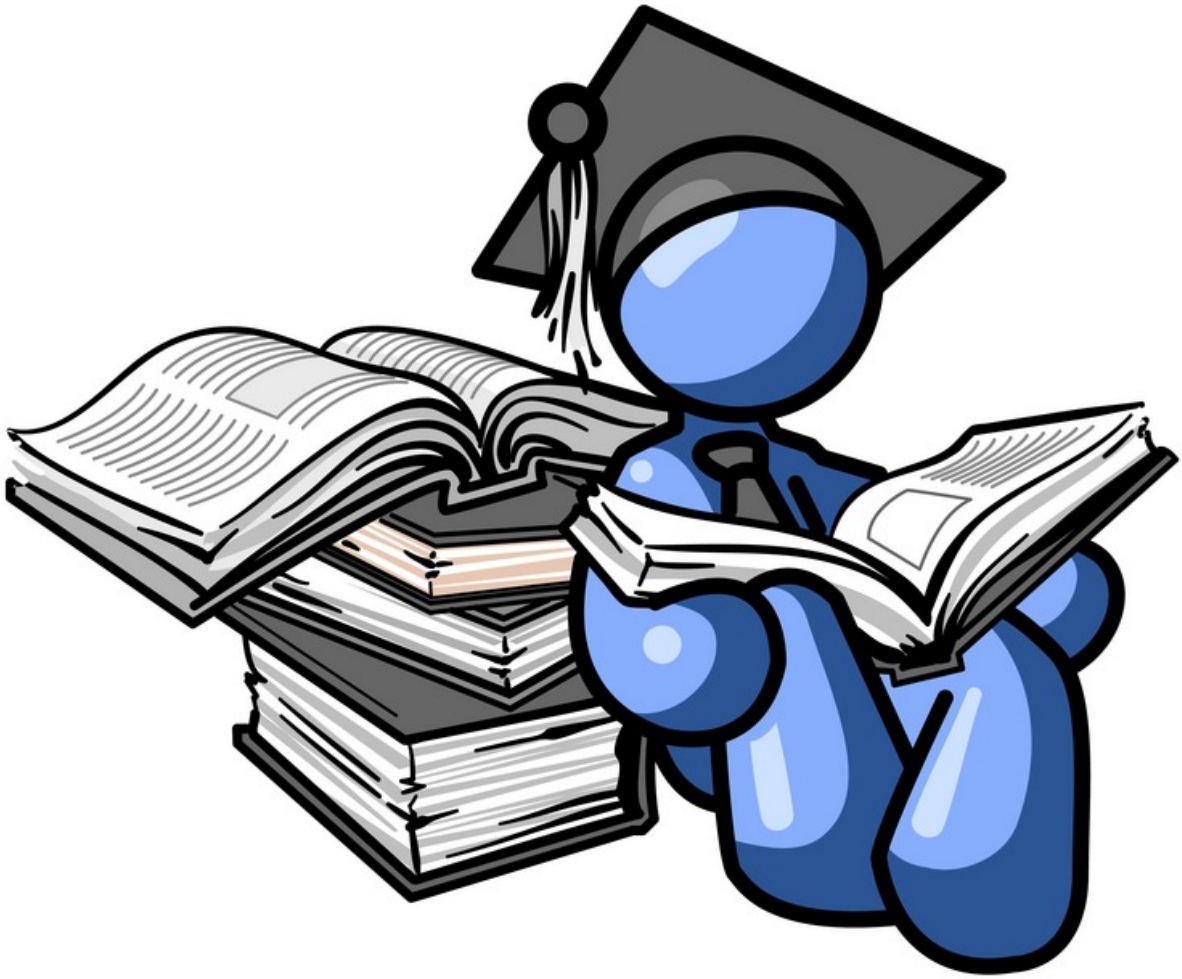


Table 8.20 Measurement of density

Group	Initial Mean density /cm.sq area (mm)	Mean density /cm.sq area After 15 days (mm)	Mean density /cm.sq area After 30 days (mm)	Mean density /cm.sq area After 60 days (mm)	Mean density /cm.sq area After 90days (mm)
Test	0.09±0.16	0.14±0.14	0.13±0.17	0.14±0.16	0.14±0.2
Control	0.08±0.17	0.07±0.16	0.08±0.18	0.11±0.10	0.11±0.14

SUMMARY AND CONCLUSION



9. SUMMARY AND CONCLUSION

❖ Phytochemical Studies

The phytochemical constant were carried out for the plants powder and extracts of L*agenaria siceraria*, *Trichosanthis cucumarina*, *Tridax procumbens* to bring the quality and purity of the valuable medicinal plants

Preliminary phytochemical screening were carried out for all the plants and its extracts to determine the presence of active principle in plants

Fluorescence analysis was carried out to detect the presence of chromophore present in the powder and extracts. No fluorescence was observed for powder as well as extracts

Selected plants powder were extracted with ethanol to bring all the active principle

Qualitative estimation of total flavonoid contend and total Phenolic content were determined by spectrophotometrically all the extract showed significant amount of flavonoid and phenolic compounds

❖ Development and Standardization of formulation

Poly herbal gel was prepared with water soluble polymer Carbopol, propylene glycol 400, povidone, triethanolamine to bring a good absorption capacity of the plant extracts on scalp.

The standardization parameters of the gel are viscosity, pH, Homogeneity, Spreadability, content uniformity, skin irritation test all were carried out to bring a quality, purity and safety of the prepared gel formulation

❖ Clinical evaluation of hair growth activity

The prepared poly herbal formulation was taken for the determination of hair growth activity of the selected plants

The clinical evaluation of prepared gel was carried on the human volunteers and compared with the reference who applied gel without the extract. The growth of hair

measured by trichoscope and the growth was completely observed after the 90 days

Hence, from these studies it is concluded that the prepared poly herbal gel containing *Lagenaria siceraria*, *Trichosanthis cucumarina*, *Tridax procumbens* proved hair growth activity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Annexure

सिद्ध केंद्रीय अनुसन्धान संस्थान

(सी.सी.आर.एस., चेन्नई, आयुष मंत्रालय, भारत सरकार)

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11th Aug 2016

CERTIFICATE

Certified that the plants/drug submitted for identification by R. Kopperundevi, M. Pharm 2nd year, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai - 600 003, are identified as

Surai	-	*Lagenaria siceraria* (Molina) Standl. (Leaves)
Kinatradi poondu	-	*Tridax procumbens* L. (Leaves)
Peipudal	-	*Trichosanthes cucumerina* L. (Leaves)

Sasikala Ethirajulu.

Sasikala Ethirajulu
Consultant (Pharmacognosy)

11/8/16

P.Sathiyarajeswaran
Assistant Director Incharge

ASSISTANT DIRECTOR I/C
CENTRAL RESEARCH INSTITUTE FOR SIDDHA
Arumbakkam, Chennai-600 106.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301A
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
R.Kopperundevi
II Year M.Pharmacy Student
Department of Pharmacognosy
College of Pharmacy
Madras Medical College
Chennai

Dear Dr. R.Kopperundevi,

The Institutional Ethics Committee has considered your request and approved your study titled **"DEVELOPMENT AND STANDARDISATION OF HERBAL GEL FOR HAIR GROWTH AND ITS CLINICAL STUDIES " NO. 31122016.**

The following members of Ethics Committee were present in the meeting hold on **14.12.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.M.K.Muralidharan,MS.,M.Ch.,Dean, MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.B.Vasanthi,MD., Prof.of Pharmacology.,MMC,Ch-3	: Member
5.Prof.A.Rajendran,MS, Prof. of Surgery,MMC,Ch-3	: Member
6.Prof.N.Gopalakrishnan,MD,Director,Inst.of Nephrology,MMC,Ch	: Member
7.Prof.Baby Vasumathi,MD.,Director, Inst. of O & G	: Member
8.Prof.K.Ramadevi,MD.,Director,Inst.of Bio-Che,MMC,Ch-3	: Member
9.Prof.R.Padmavathy, MD, Director,Inst.of Pathology,MMC,Ch-3	: Member
10.Prof.S.Mayilvahanan,MD,Director, Inst. of Int.Med,MMC, Ch-3	: Member
11.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
12.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
13.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

68th INDIAN PHARMACEUTICAL CONGRESS

ipc 2016 VISAKHAPATNAM

Theme Quality Pharmaceuticals and Patient Welfare

PHARMA VISION 2020 — Swasth Bharat — PHARMACISTS FOR A HEALTHY INDIA

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. KOPPERUNDEVI

of MADRAS MEDICAL COLLEGE

has attended the 68th IPC as Registered Delegate held at AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P. during 16th – 18th December 2016.

Mr. S.V. Veerramani	Dr. Rao Vadlamudi	Dr. T.V. Narayana	Dr. G. Nagarjuna Reddy
President, IPCA-2016	LOC, Chairman	LOC, Secretary	Chairman, Reg. Com.

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Venue: University Collage of Pharmaceutical Sciences

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69, Anna Salai, Guindy, Chennai - 600 032.

CME

on

"PATENCY"

CATEGORY - II

CREDIT POINTS - 5

Certificate of Participation

This is to certify that ~~Dr.~~ / ~~Mr.~~ / ~~Mrs.~~ / Ms. KOPPERUNDEVI. R.

has participated as a ~~Speaker~~ / Participant in the **CME on "PATENCY"** *conducted by the Department of Medical Education and Curriculum Development, The Tamilnadu Dr.M.G.R. Medical University, Chennai on 26th August 2016.*

Head of the Department
Department of Medical Education &
Curriculum Development

REGISTRAR (FAC)

VICE-CHANCELLOR

Sri Ramachandra University

31 Years SRMC & RI 1985-2016

[Declared under Section 3 of the UGC Act, 1956]
[Accredited by NAAC with 'A' Grade]

International Conference on
"Clinical Pharmacy Practice Skills - Recent Perspectives"

7TH - 9TH SEPTEMBER 2016

THIS IS TO CERTIFY

DR/MR/MS KOPPERUN DEVI. R HAS

ATTENDED AS

~~RESOURCE PERSON~~/~~ORAL~~ PRESENTER/DELEGATE IN THE

CONFERENCE ORGANIZED BY THE

DEPARTMENT OF PHARMACY PRACTICE,

FACULTY OF PHARMACY, SRI RAMACHANDRA UNIVERSITY,

PORUR, CHENNAI–600116.

THIS CERTIFICATE CARRIES 12 CREDIT POINTS

DR P SEENIVASAN
ORGANIZING SECRETARY

DR D CHAMUNDEESWARI
CONVENOR

DR K V SOMASUNDARAM
DEAN OF FACULTIES

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PALLAVARAM - CHENNAI - INDIA

VELS UNIVERSITY

CERTIFICATE

~~Dr.~~/~~Mr.~~/Ms./ R. KOPPERUNDEVI

of Madras Medical College, Chennai

has participated as delegate/~~organizer~~/~~resource person~~ in the one day workshop on "Systematic Review on Preclinical Studies" on 28th January 2016

Vice Chancellor

Registrar

Convener

RTIP-2016
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Certificate

This is to certify that ~~Dr/Mr~~/Ms. KOPPERUNDEVI . R
of M. Pharm, COP - MMC
participated in the 5th National seminar on "Recent Trends in Industrial Pharmacognosy - 2016" Organised by Department of Pharmacognosy, College of Pharmacy, MTPG & RIHS, on 19th March 2016, at Puducherry and presented a paper in the Oral/Poster/e-Poster session titled
.....
with co-authors.....

V. Gopal
PROF. DR. V. GOPAL
REGISTRAR ACADEMIC
CONVENOR - RTIP'16

R
Dr. R. MURALI
DEAN - MTPG & RIHS
CHIEF PATRON - RTIP'16

RTIP'16

ACCREDITED BY THE TAMILNADU Dr M.G.R MEDICAL UNIVERSITY, CHENNAI WITH 05 CREDIT POINTS